VOLUME 46 OCTOBER, 1956 NUMBER 10

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

UNIVERSITY OF MICHIGAN
NOV 1 1956
SCIENCE LIBRARY

CONTENTS

Inverse pitting in xyloporosis and tristeza — I. Reichert and E. Winocour 527
The effect of placement depth on the activity of Nemagon — J. D. Gilpatrick, S. T. Ichikawa, Mason Turner, and C. W. McBeth 529
Allyl alcohol as a soil fungicide — Amegda J. Overman and D. S. Burgis 532
A decline of sour cherry caused by a virus of the little cherry type — J. A. Milbrath and H. E. Williams 535
Transmission of factors for urediospore color in Puccinia graminis var. tritici by means of nuclear exchange between vegetative hyphae — R. R. Nelson 538
Cross protection with two rust fungi — C. E. Yarwood 540
Black shank resistance in flue-cured tobacco as influenced by tolerance to certain parasitic nematodes — E. L. Moore, P. N. Drolsom, F. A. Todd, and E. E. Clayton 545
Site of disease resistance induced by plant-growth regulators in tomato — David Davis and A. E. Dimond 551
Origin and composition of cell inclusions associated with certain tobacco and crucifer viruses — Miguel Rubio 553
Suscepts, indicators, and filters of tristeza virus, and some differences between tristeza in Argentina and in Florida — L. C. Knorr 557
Upper altitudinal limits of lodgepole pine dwarfmistletoe in the central Rocky Mountains — Frank G. Hawksworth 561
Use of shade tent and insecticides in studies of virus spread — Paul E. Waggoner and James B. Kring 562
New races of Colletotrichum lindemuthianum in Mexico — William D. Yerkes, Jr., and Moises Teliz Ortiz 564
Antibiotics against plant disease. I. Laboratory and greenhouse survey — Thomas G. Pridham, Lloyd A. Lindenfelser, Odette L. Shotwell, Frank H. Stodola, Robert G. Benedict, Colette Foley, Richard W. Jackson, William J. Zaumeyer, William H. Preston, Jr., and John W. Mitchell 568
Antibiotics against plant disease. II. Effective agents produced by Streptomyces cinnamomeus forma azacoluta f. nov. — Thomas G. Pridham, Odette L. Shotwell, Frank H. Stodola, Lloyd A. Lindenfelser, Robert G. Benedict, and Richard W. Jackson 575

Phytopathological Notes:

A method for observing stomatal penetration by uredospore germ tubes of Puccinia graminis f. sp. tritici — Lawrence J. Petersen 581
Rediscovery of sulfur as a fungicide — S. E. A. McCallan 582
Symptomatology of bacterial spot of pepper and tomato in southern Florida — R. S. Cox, Robert A. Conover, and Grover Sowell, Jr. 582
The effects of temperature on the resistance to oat stem rust conditioned by the BC genes — B. J. Roberts and M. B. Moore 584

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

KARATHANE controls powdery mildews on fruits, cucurbits, ornamentals

What other mildews can **KARATHANE** *control?*

Frankly, we don't have all the answers. We invite phytopathologists and other scientists to obtain some of the information by including KARATHANE in their research programs.

We know this much: In only two years of commercial use KARATHANE WD has won world-wide acceptance for powdery mildew control. It has brought severe outbreaks under control in apple areas of this country. It is now successfully combatting powdery mildew on pears, cucurbits, peaches, cotton, tobacco, roses, either in the United States or overseas.

Since KARATHANE has proven so effective in controlling representative species of powdery mildew you will want to include it whenever you look into powdery mildew control.

KARATHANE is an unusual pesticide. It is one of the few commercially available fungicides which also controls certain species of mites.

KARATHANE is a trade-mark, Reg. U. S. Pat. Off. and in principal foreign countries.

Chemicals for Agriculture

ROHM & HAAS COMPANY

WASHINGTON SQUARE, PHILADELPHIA 5, PA.

Representatives in principal foreign countries

INVERSE PITTING IN XYLOPOROSIS AND TRISTEZA [1]

I. Reichert and E. Winocour[2]

SUMMARY

A form of bark pitting found in Israel on citrus trees that have also the typical symptoms of the disorder originally described as xyloporosis were studied histologically. Comparisons with similar forms of pitting described in California, Florida, and Argentina on citrus trees affected by the tristeza (quick decline) virus disease and on trees suffering from other disorders of unknown cause show that this type of bark pitting is not specific for any single virus or cause. This indicates that it may not be a safe procedure to make specific diagnoses on the basis of this particular symptom on citrus.

A peculiar type of bark and wood abnormality appears on xyloporosis-affected Shamouti sweet orange budded to Palestine sweet lime rootstock trees in Israel. It consists of small projections on the cambial face of the wood; these fit into small pin holes on the corresponding cambial face of the bark. This phenomenon is somewhat the reverse of the usual xyloporosis type of pitting, which is characterized by small projections of another type on the cambial face of the bark that fit into corresponding pits in the wood (5). The reverse pitting abnormality on xyloporosis-affected trees has been termed "inverse xyloporosis" (6). On the affected Shamouti sweet orange budded to Palestine sweet lime rootstock, this type of pitting has been observed mainly at or above the bud union. Less frequently, it has been noted on the rootstocks of diseased Shamouti on Sweet lime, Rough lemon, and Trifoliate orange (6); in 1 instance, it was noted on a sour orange inarch to Shamouti on Palestine sweet lime rootstock.

Schneider (8, 10) has described this type of bark pitting on lemon trees declining from a disorder (of unknown cause) that he designated sour orange rootstock necrosis. He also observed the same symptom on tristeza-affected trees of sweet orange on sour orange rootstock (9). Busby in Florida (1) observed a "pinholing" or "sieve-like appearance" in the cambial surface of the bark immediately below the bud union of trees affected by tristeza. DuCharme and Knorr (3) described the same symptom on sweet orange trees on various tristeza-susceptible rootstocks in Argentina and gave to it the name "honeycombing". Cohen and Knorr (2) described "honeycombing" as a macroscopic symptom of tristeza in Florida, but they reported that similar symptoms have been found on citrus trees that are not infected with the virus of tristeza.

In view of the speculation in recent years on a possible relationship between xyloporosis and tristeza, and the fact that a morphologically identical pitting symptom is associated with both diseases, it seemed desirable to undertake a comparative histological study of the "inverse xyloporosis" and "honeycombing" symptom expressions. For this purpose, a radial section (Fig. 1, A) of bark across the bud union of a tristeza-affected sweet orange on sour orange stock that showed "honeycombing" below the bud union was obtained through the courtesy of Dr. M. Cohen of Gainesville, Florida, and compared to similar radial sections of bark across the bud union (Fig. 1, B) of xyloporosis-affected sweet orange on Palestine sweet lime in Israel. In the latter group of trees, the "inverse xyloporosis" abnormality appeared above the bud union. In addition, transverse sections (Fig. 1, E) of Shamouti scion bark and wood showing "inverse xyloporosis" were also examined. At the time of this study, transverse sections of the Florida "honeycombing" abnormality were not available. Consequently, use was made of a photomicrograph of a transverse section (Fig. 1, D) published by DuCharme and Knorr (3).

The histological examination of the "inverse xyloporosis" abnormality in the tissues of the Shamouti sweet orange scion has revealed that the ray cells of both the xylem and the inner phloem adjacent to the cambium have undergone hyperplasia and lignification (Fig. 1, B, E). Thus this form of bark pitting appears to be identical with the "honeycombing" or "pinholing" abnormality found on tristeza-affected trees (Fig. 1, A, D).

Schneider (9), who described the hyperplastic phloem ray phenomenon as an anatomical symptom of quick decline in diseased sour orange stock combinations in California, has stated: "Below the bud union the rays occasionally become hyperplastic. Cells of the hyperplastic rays may develop secondary walls, and the walls may become lignified. When the bark is removed, such woody hyperplastic rays may remain attached to the xylem; small holes therefore occur on the cambial face of the bark." A photomicrograph published by Schneider (9) of the hyperplastic ray phenomenon, as seen in radial section, is reproduced here (Fig. 1, C) for comparison; the histological similarity to the radial sections of "inverse xyloporosis" (Fig. 1, B) can be readily noted. From the descriptions of the "pin-holing" effect on the bark of the

[1] Accepted for publication April 26, 1956.
Publication of the Agricultural Research Station, Rehovot, Series No. 123.

[2] Respectively, Head of Division of Plant Pathology, and Pathologist, Agricultural Research Station, Rehovot, Israel.

sour orange rootstock of lemon trees declining from sour orange rootstock necrosis (8, 10), it is evident that in this instance also hyperplasia and lignification of the medullary ray cells are associated with this symptom. DuCharme and Knorr, (3) in a brief report on the histology of "honeycombing" in Argentina, have stated that "histological examination shows the pegs to consist of hypertrophied medullary rays." A photomicrograph of a transverse section published by these authors is reproduced here (Fig. 1, D) so that its similarity to the transverse section of "inverse xyloporosis" (Fig. 1, E) can be noted.

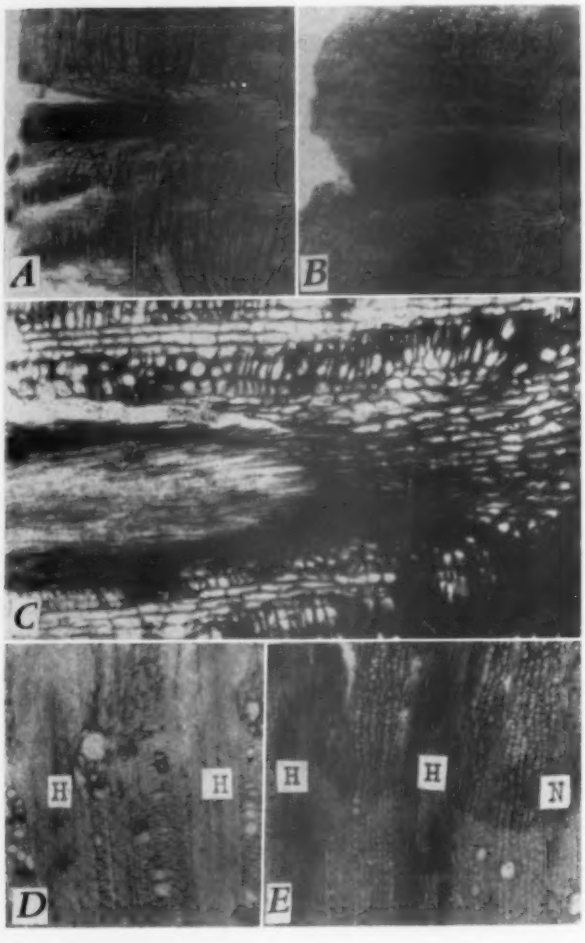


FIG. 1. Comparative histology of bark pitting on xyloporosis and tristeza-affected citrus trees. A) Radial section of bark, just below bud-union, of tristeza-affected sour orange rootstock in Florida. Note the hyperplastic and lignified phloem ray. Cambium at left. (×70). B) Radial section of bark, just above bud union, of xyloporosis-affected Shamouti sweet orange scion in Israel. Note the similar hyperplastic and lignified phloem ray. Cambium at left. (×70). C) Radial section of bark, below bud union, of quick-decline-affected sour orange rootstock in California, showing similar hyperplastic development of a phloem ray. Cambium at left. Reproduced from Schneider (9). (×100). D) Transverse section of bark and wood showing the "honeycombing" abnormality on Pernambuco grapefruit rootstock in Argentina. Note the hyperplastic and lignified rays (H) in both the inner phloem and outer xylem. Reproduced from DuCharme and Knorr (3). E) Transverse section of xyloporsis-affected Shamouti scion bark and wood in Israel. Note similar hyperplastic and lignified rays (H) of both inner phloem and outer xylem. Normal ray at N (×70).

From the above it is evident that no differences are apparent in the histology of the forms of inverse pitting characteristic of xyloporosis in Israel, of tristeza in Florida, and of quick decline and sour orange rootstock necrosis in California. This might appear to indicate that the reactions of certain citrus tissues to the principal causal agents of xyloporosis and tristeza are the same and thus strengthen the view point of those (4) who have in the past suggested an identity between the 2 diseases. Conversely, Cohen and Knorr (2) have reported: "However, a range of conditions has been found in bark of sweet orange, rough lemon, sweet lemon, Tahiti lime, and other citrus species, not necessarily at the bud union, that stimulate honeycombing to a greater or lesser degree. These may be distinguished from typical tristeza honeycombing by the absence of other histological features of tristeza and by failure to transmit vein clearing symptoms of tristeza to Key lime seedlings when budded from affected trees." This finding, that inverse pitting forms have appeared in Florida on citrus species that do not test positive for tristeza, tends to weaken the suggestion identifying xyloporosis with tristeza. Other factors relating to the question of an identity between the 2 diseases have been summarized elsewhere (7).

It appears that "honeycombing" or "inverse xyloporosis" is not a rigidly confined tissue reaction of certain species to specific virus infections. In fact, the possibility remains that such symptoms may not always be attributable to virus infections. In this connection, further studies on the developmental histology of the different morbid pitting forms characteristic of some virus-induced decline diseases in citrus may aid in the clarification of the complex relationships between such diseases. Presently, at least, it may not be a safe procedure to make specific diagnoses on the basis of this particular symptom on citrus trees.

DIVISION OF PLANT PATHOLOGY
AGRICULTURAL RESEARCH STATION
REHOVOT, ISRAEL

LITERATURE CITED

1. BUSBY, J. N. 1953. Tristeza in Florida. Citrus Industry **34**(8): 5-7.
2. COHEN, M., AND L. C. KNORR. 1954. Honeycombing—a macroscopic symptom of tristeza in Florida. (Abs.) Phytopathology **44**: 485.
3. DUCHARME, E. P., AND L. C. KNORR. 1954. Vascular pits and pegs associated with diseases in citrus. U.S. Dept. Agr. Pl. Dis. Rptr. **38**: 127-142.
4. MCCLEAN, A. P. D. 1950. Possible identity of three citrus diseases. Nature [London] **165**: 767-768.

5. REICHERT, I. 1955. New light on tristeza and xyloporosis in citrus. 14th Internatl. Hort. Cong. Proc., Amsterdam. (In press.)
6. REICHERT, I., AND J. PERLBERGER. 1934. Xyloporosis, the new citrus disease. Hadar **7**: 163-167, 172, 193-202.
7. REICHERT, I., I. YOFFE, AND A. BENTAL. 1953. Shamouti orange on various rootstocks and its relation to xyloporosis. Pal. Jour. Bot. **7**: 163-184 (Rehovot series).
8. SCHNEIDER, H. 1952. Necrosis of sieve tubes below bud union of lemon trees on sour orange rootstocks. Citrus Leaves **32**(3): 10-11, 35, 37.
9. SCHNEIDER, H. 1954. Anatomy of bark of bud-union, trunk, and roots of quick decline-affected sweet orange trees on sour orange rootstock. Hilgardia **22**: 567-581.
10. SCHNEIDER, H. 1955. Decline of lemon trees on sour orange rootstock. Citrus Leaves **35**(12): 10-11, 28-29.

THE EFFECT OF PLACEMENT DEPTH ON THE ACTIVITY OF NEMAGON[1]

J. D. Gilpatrick, S. T. Ichikawa, Mason Turner, and C. W. McBeth

SUMMARY

Liquid Nemagon injected 2 in. deep in sandy soil gave almost complete control of the root knot nematode, *Meloidogyne incognita* var. *acrita* Chitwood, 1949 in the surface 12 in. Placement 4 in. deep controlled this nematode down to 18 in. Deeper placement was required for deeper penetration, but injections at 10 and 12 in. failed to control a few nematodes near the surface. For most purposes a placement 6–8 in. deep is recommended for both deep penetration and surface cleanup. At application depths of 4–8 in., Nemagon impregnated on Attaclay granules gave as good control in the upper 24 in. of soil as did the liquid. In the 24 to 36 in. zone, the liquid was slightly more effective than the granules.

Most recommendations that appear in the literature advocate an application depth of 6–8 in. for the volatile nematocidal soil fumigants (2, 3, 9, 10); however in certain special cases, application depths greater than 8 in. have been suggested for nematocides. Foote (4) recommended that high dosages of D-D (1,3-dichloropropene; 1,2-dichloropropane), ethylene dibromide (1,2-dibromoethane), and carbon bisulfide be applied 12–15 in. deep for best control of the citrus nematode. Baines and Clark (1) also recommended that high dosages of D-D be injected at a depth of 12–15 in. for control of this nematode.

Raski and Allen (7) used an 8-in. depth for over-all[2] treatments with D-D and ethylene dibromide for the control of root knot of cotton, but they injected 13 in. below the top of the bed for row treatments to minimize the possible loss of fumigant from the side the bed.

Despite a lack of experimental data, the authors believe, from their own experiences and those of other investigators, that the recommended application depth of 6-8 in. probably produces optimum nematode control in most cases for D-D and ethylene dibromide.

Since Nemagon is less volatile (boiling point, 196°C at 760 mm) than is D-D or ethylene dibromide, it seemed possible that depth of application might not be as critical for Nemagon as it is believed to be for the more volatile materials. Thus the following experiments were conducted in an attempt to determine the effect of application depth on the activity of this new nematocide.

HAND-GUN INJECTION OF LIQUID NEMAGON.—This experiment was conducted in a field of uniform sandy loam (moisture equivalent 4.3 per cent) that was heavily infested below the 4-in. level with a root knot nematode, *Meloidogyne incognita* var. *acrita* Chitwood, 1949. The nematode population in the surface 4 in. was relatively low as indicated by the low root knot indices of soil samples taken 0–6 in. below the surface in these experiments and by previously reported profile studies (5) that were conducted in this same field.

Plots 7 × 13 ft. were given an over-all treatment with liquid Nemagon at the rate of 1 gal. per acre with a hand-gun injector by methods previously described by McBeth and Bergeson (6). Nemagon was diluted with Shell Lacquer Diluent A (petroleum naptha in boiling range 54–82°C) so that the diluted material was injected at the rate of 10 gal. per acre. At the time of treatment, the soil moisture was 5.6 per cent of the oven-dry weight and the soil temperature at a depth of 8-in. was 62°F.

Four randomized blocks were used, each containing an untreated plot and 6 plots treated with Nemagon at depths of 2, 4, 6, 8, 10, and 12 in. For the 2- and 4-in. applications, a special shield was attached to the

[1] Accepted for publication May 3, 1956.
Nemagon, a trademark of the Shell Chemical Corporation, is 1,2-dibromo-3-chloropropane, formerly known as Shell OS 1897.
[2] The authors believe that the term "over-all" is to be preferred to "solid," "flat," or "broadcast" for describing a complete treatment in which the spacing between injections is usually 12 in.

TABLE 1.—*Root knot indices*[a] *of squash plants grown in plots treated with liquid Nemagon injected by hand-gun at different depths*

Depth of injection (in.)	Indices of indicated replicates A	B	C	D	Mean index
2	0	0	0	0.2	0.05
4	0.3	0	0	0	0.075
6	0	0	0	0.4	0.1
8	0	0.2	0	0	0.05
10	1.1	1.4	2.7	0.9	1.53
12	0.5	5.1	3.2	7	3.95
Untreated	52.5	67.5	40.5	57	54.4

[a] 0 = No infection, 100 = maximum infection.

hand gun at the ground level to assure accurate injection depths. The plots were watered with an overhead irrigation system throughout the experiment to minimize cross contamination from plot to plot.

The degree of control of the root knot nematode at each depth of application was determined by 2 methods: 1) by planting the plots directly to an indicator crop and 2) by taking soil samples from the plots and sowing these to an indicator crop in the greenhouse. In the first method, hereafter referred to as the direct-seed assay, the plots were sown to yellow crookneck squash (*Cucurbita pepo* L.) in rows 12 in. apart, 2 weeks after treatment. Approximately 2½ months later, 100 plants were dug from each plot and the roots graded for severity of root knot by visual estimation according to methods described previously (5, 6). Root knot indices were calculated by a method similar to that described by Smith and Taylor (8).

In the second method, referred to as the soil-sample assay, cores of soil of 2-in. diameter were taken in duplicate from each plot 8 weeks after treatment and separated into successive 6-in. layers down to a depth of 36 in. Each 6-in. sample was sown to tomatoes in the greenhouse, and the resulting degree of nematode infection of the tomato roots was determined after 6 weeks.

From the results of the direct-seed assay (Table 1), it would appear that Nemagon was equally effective at all injection depths of 2, 4, 6, and 8 in. Depths of 10 or 12 in. were slightly less effective; however, when based on soil samples (Table 2), control was apparently optimum with injections of 8–12 in. for the entire 36 in. sampled. In the top 18 in., control, as measured by sampling, was similar to that measured by direct-seed assay (Table 1), in which plant roots were dug to a depth of 12 in.

The soil-sample assay indicated that shallow injections (2–4 in.) gave excellent control in the top 18 in. of soil but did not allow Nemagon to penetrate effectively below that depth. The deeper the injection, the better the control below 18 in., with best control from a placement of 12 in. The 10- and 12-in. injections, however, failed to kill a few nematodes at the surface. This no doubt accounts for the slight infections of the squash plants used for the direct-seed assay of plots injected at these depths.

TABLE 2.—*Root knot indices*[a] *of tomato plants grown in soil samples taken at 6-in depths from plots treated with liquid Nemagon injected by handgun at different depths*

Depth of sample (in.)	Indices for indicated depth of injection (in.) 2	4	6	8	10	12	Untreated
0–6	0	0	0	0	3.5	1.5	45
6–12	0	0	0	0	0	1.5	100
12–18	0	0	2	0	0	0	86
18–24	14.5	12.5	2	3.5	0	0	86
24–30	52	39.5	12.5	2	3.5	0	79
30–36	57	53.5	35.5	7.5	3.5	0	71
0–36 (mean)	20.8	17.6	8.7	2.2	1.8	0.5	78

[a] All values given are the average of 2 replicates; 0 = no infection, 100 = maximum infection.

MACHINE APPLICATION OF ATTACLAY AND LIQUID FORMULATIONS OF NEMAGON.—Nemagon impregnated on Attaclay[3] granules was compared with a liquid formulation at different injection depths. Both materials were applied over-all at the rate of 1.25 gal. (21.6 lb.) of Nemagon per acre. The Attaclay formulation was applied with a Gandrud Fertilizer Distributor (Gandy Spreaderette) especially adapted with chisels. Since this formulation contained 25 per cent Nemagon by weight, a total of 86.4 lb. was applied per acre. Liquid Nemagon diluted 1:7 with Diluent A was injected at the rate of 10 gal. of formulated material per acre with conventional tractor-mounted equipment. The chisels of both applicators were spaced 12 in. apart.

The depths of placement for both formulations were 0, 2, 4, 6, and 8 in. There were 2 replicated 5 × 40-ft. plots for each depth of Attaclay formulation and 1 plot only of the same size for each depth of the liquid formulation.

These plots were located adjacent to those of the previous hand-gun experiment. The soil temperature at the 8-in. depth was 80°F at the time of treatment, and the moisture content was 5.2 per cent of the oven-dry weight of soil. Nematode control was assayed by the same 2 methods used in the first experiment.

Results of the direct-seed assay (Table 3) indicated

[3] Attaclay, a product of the Attapulgus Clay Co., is a complex hydrated aluminum magnesium silicate.

TABLE 3.—*Root knot indices of squash plants grown in plots treated with granular and liquid Nemagon injected by machine at different depths*

Depth of injection (in.)	Indices[a] with Attaclay formulation	Indices[a] with liquid formulation
0	65	67
2	23	0
4	1.5	0.15
6	0	0
8	0	0
Untreated	86	96

[a] 0 = No infection.

TABLE 4.—*Root knot indices of tomato plants grown in soil samples taken at 6 in. depths from plots treated with granular and liquid Nemagon injected by machine at different depths*

Depth of sample (in.)	Indices[a] with granules for depth of application (in.) indicated						Indices[a] with liquid for depth of injection (in.) indicated					
	0	2	4	6	8	Untreated	0	2	4	6	8	Untreated
0–6	0	0	0	0	0	3.5	0	0	0	0	0	0
6–12	0	0	0	0	0	7	14.5	0	0	0	0	21.5
12–18	0	7	0	0	0	10.5	14.5	0	0	0	0	28.5
18–24	7	18	0	0	0	14.5	28.5	0	0	0	0	28.5
24–30	14	18	7	0	0	14.5	14.5	0	0	0	0	14.5
30–36	14	18	18	3.5	3.6	28.5	7	7	0	0	0	28.5
0–36 (mean)	5.8	10.2	4.2	0.6	0.6	13.1	12.9	1.2	0	0	0	20.3

[a] 0 = No infection.

that both the Attaclay and the liquid formulations gave satisfactory nematode control in the surface foot of soil when applied at depths of 4–8 in. When application was at a depth of 2 in., only the liquid gave control in the surface foot. The soil-sample assay (Table 4) indicated that at any given application depth the liquid controlled nematodes at greater depths than did the granules. Nevertheless, excellent control down to 24 in. was obtained with the Attaclay granules at placements of 4–8 in.

The soil-sample assay demonstrated deep penetration of the liquid formulation with all placements. In the previous hand-gun plot, such deep penetration with placements of 2–6 in. was not obtained. This difference may be related to the higher nematode population in the hand-gun plot or to the slightly higher dosage used in the plots applied by machine (1 gal. versus 1.25 gal.).

DISCUSSION.—The recommended depth of placement of Nemagon depends on the depth to which nematode control is required. The 2 experiments described here indicated that injection of Nemagon 2 in. deep gave satisfactory control of the root knot nematode in the surface foot of soil. Deeper injection depths were required for deeper penetration; however, when the depth of injection was increased to 10 or 12 in., control near the surface was slightly reduced. For most purposes, a placement 6–8 in. deep probably would be best, for it would give good penetration and surface cleanup. For shallow-rooted crops, where nematode control in the top 12–18 in. is desired, shallow placement (2–4 in.) seems adequate. This would appear to be a unique property of Nemagon, since it is generally believed that other more volatile soil fumigants (D-D or ethylene dibromide) are not efficient when applied at these depths. Even for shallow-rooted crops, however, the 8-in. depth would be preferable unless ease of application is a limiting factor. With deep-rooted annuals and perennials, where deep penetration is desired, and especially with tree and vine crops where high dosages are likely to be used, deep applications (10–12 in.) are probably advisable.

Nemagon formulated on Attaclay was almost as effective as the liquid. Results from other locations also indicate that liquid or granular Nemagon injected 6–8 in. deep gave similar responses. Thus it seems likely that Nemagon may be applied with fertilizer or granular-insecticide applicators that are capable of placing the material at the recommended depths. This would be of a decided advantage, since most growers have available equipment of this type or equipment that may easily be adapted, but many do not have access to equipment needed for injection of liquid soil fumigants.

These tests illustrate the advantage of using a combination of the direct-seed and the soil-sample indicator-crop techniques for assaying for nematode control. The first method, which is probably adequate for shallow-rooted crops, measures the amount of control in the top 12 in. of soil at the termination of the experiment. The soil-sample assay gives the degree of control at various depths in the soil at the time of sampling.

These tests were conducted in light sandy soil and at limited dosages. Variations of these might modify the conclusions drawn here.

NEMATOLOGY DEPARTMENT
SHELL DEVELOPMENT COMPANY
AGRICULTURAL RESEARCH DIVISION
MODESTO, CALIFORNIA

LITERATURE CITED

1. BAINES, R. C., AND O. F. CLARKE. 1951. Citrus-root nematode. Calif. Citrog. **37**: 60, 86.
2. CHRISTIE, J. R. 1947. Soil fumigation for control of nematodes and other soil-inhabiting organisms. U. S. Dept. Agr. Pl. Dis. Rptr. Sup. 170: 169-189.
3. CHRISTIE, J. R., AND A. L. TAYLOR. 1952. Controlling nematodes in the home garden. U. S. Dept. Agr. Farmers' Bul. 2048, 11 p.
4. FOOTE, F. J. 1952. Soil fumigation for citrus replanting. Calif. Citrog. **37**: 276, 286-288.
5. ICHIKAWA, S. T., J. D. GILPATRICK, AND C. W. MCBETH. 1955. Soil diffusion pattern of 1,2-dibromo-3-chloropropane. Phytopathology **45**: 675-678.
6. MCBETH, C. W., AND G. B. BERGESON. 1953. Methods of assaying nematocides. Phytopathology **43**: 264-267.
7. RASKI, D. J., AND M. W. ALLEN. 1953. Control of root-knot nematode on cotton. U.S. Dept. Agr. Pl. Dis. Rptr. **37**: 193-196.
8. SMITH, A. L., AND A. L. TAYLOR. 1947. Field methods for testing for root-knot infestation. Phytopathology **37**: 85-93.
9. TAYLOR, A. L. 1951. Chemical treatment of the soil for nematode control. Adv. in Agron. **3**: 243-264.
10. THORNE, G. 1952. Control of the sugar beet nematode. U.S. Dept. Agr. Farmers' Bul. 2054, 18 p.

ALLYL ALCOHOL AS A SOIL FUNGICIDE [1]

Amegda J. Overman and D. S. Burgis [2]

SUMMARY

Allyl alcohol, used as a preplanting drench to control weeds on the sandy soils of vegetable seedbeds, was highly effective in controlling damping-off organisms. Microbiological studies of treated and nontreated plots indicated that *Trichoderma* spp. were increased whereas other fungi that are capable of developing on peptone-agar plates were reduced significantly. Five seasons' data involving various seedbed conditions have shown consistently that high populations of Trichoderma were present for 8 weeks after an application of 25 gal. per acre of allyl alcohol.

Moderate soil temperatures and the presence of living hosts during the entire year make control of seedbed pests in the sandy soil of Florida a constant problem. Workers interested in improvement of crop management have screened many chemicals in attempts to develop preplanting soil treatments effective against weeds, nematodes, and soil-inhabiting phytopathogenic organisms. Among these materials, allyl alcohol has attracted increasing interest. The material first came into use as an herbicide. In recent years its use as a pre-emergence herbicide has been recommended for tobacco-plant beds in North Carolina (2), and additional work has been done with the same crop in West Virginia (9). Emmert and Klinker (11) found allyl alcohol superior to calcium cyanamid in controlling weeds in plantings of snapbeans and sweet corn. In pine-seedling nurseries, Lindgren and Henry (14) found the material to be an efficient weed- and grass-seed toxicant. Although it was not as effective as chlorpicrin (trichloronitromethane) in controlling the root-rot organisms present in the beds, the largest number of seedlings, "both total and plantable," were produced in plots treated wtih allyl alcohol as a drench at the rate of 360 lb./acre.

Preliminary data obtained in 1949 at the Gulf Coast Experiment Station indicated that the herbicidal usefulness of allyl alcohol is limited, since it does not effectively control nutgrass (*Cyperus rotundus* L.), which infests a great part of the older cultivated land in Florida (7). It gave such excellent control of damping-off in vegetable seedbeds, however, that it was included in a long-term seedbed trial involving other herbicides, soil fungicides, and nematocides (4, 5, 6, 7, 8).

The ultimate criterion in judging the value of the soil pesticides used in this trial was crop response. Microbiological data were correlated with crop response to give a more detailed picture of the effects of the treatments. An abstract covering this work has been published (15).

MATERIALS AND METHODS.—In the preliminary work, allyl alcohol at the rate of 25 gal. per acre applied at a 1:200 dilution was the optimum dosage for damping-off control in the light sandy soils typical of the Florida West Coast area. In the long-term experiment here reported, certain plots in subirrigated groundbeds received this preplanting treatment before each of 5 consecutive growing seasons. The soil, a Leon fine sand of pH 4.8–5.3, was prepared properly for seeding (3) and drenched with allyl alcohol solution. Tomatoes were sown in these permanent seedbeds on the seventh day after application of the material. No detrimental effects were observed that would indicate toxic residues in the soil.

In order to measure the effect of allyl alcohol on plant production where nutgrass was not a serious problem, a second experiment involving dosages of 2, 7, 12, and 25 gal./acre was set up on another tract of Leon fine sand. The range of the soil pH was 5.5–6.5. Tomatoes were sown 24 hours after soil treatment.

Microbiological studies of soil samples collected weekly from the seedbed plots were used in determining and interpreting some of the changes in soil microflora brought about by the allyl alcohol treatment. Information on populations of fungi, bacteria, actinomycetes, and nematodes was obtained, by methods developed by Fred and Waksman (12), and correlated with crop data.

RESULTS.—On the permanent seedbed, plant response to soil treatment with allyl alcohol varied considerably from season to season. Two important factors were represented in this variation: nutgrass and nematode populations. The seedbed area was initially heavily infested with nutgrass. As the experiment progressed, nutgrass populations increased in the plots treated with allyl alcohol and competed with the tomato seedlings for available nutrients. This extraordinary production of nutgrass may have been a result of partial sterilization of the soil by allyl alcohol. Organisms that normally attack nutgrass may have been reduced. Where nutgrass was not a problem, a statistically significant increase in number and quality of transplants was produced by allyl alcohol applied at the rate of 12 gal. per acre; at lower dosages, increases were not statistically significant.

In some seasons, nematodes reduced the quality of seedlings. Although the general color and vigor of the roots was good, root-knot galling was in some plantings more prevalent on plants grown in soil treated with allyl alcohol than on those grown in nontreated

[1] Accepted for publication May 9, 1956.

[2] Assistant in Soils Chemistry and Assistant Horticulturist, respectively, Florida Agricultural Experiment Station.

plots. This limitation in efficiency of the material as a seedbed drench has been circumvented by combining it with a nematocide. Allyl alcohol was found to be compatible with ethylene dibromide (1,2-dibromoethane), with D-D (1,3-dichloropropene; 1,2-dichloropropane) and with Nemagon (1,2-dibromo-3-chloropropane). The combinations have exhibited the full fungicidal effectiveness of the alcohol and the full nematocidal effectiveness of the nematocides.

In other experiments where allyl alcohol treatments were compared with various fungicides, it was found that the 7-day waiting period is not necessary. Celery, tomato, pepper, cabbage, eggplant, and cucumber have not shown deleterious effects when planted 24 hours after application of the allyl alcohol.

Nitrifying bacteria, as measured by nitrification of ammonia nitrogen, were inhibited for 3 weeks after the allyl alcohol drench was applied. Actinomycete populations apparently were unaffected by the material at the rates used.

The extent to which the allyl alcohol affected soil fungi was revealed in dilution-plate counts on peptone agar. Counts of colonies of *Fusarium* spp. proved to be indicative of the general level of pathogenic fungi in the treated soil and correlated inversely with numbers of transplantable plants produced. In each of the 5 seasons, biometric analyses supported the observation that Fusaria were reduced significantly by allyl alcohol and by a combination of allyl alcohol and ethylene dibromide (Table 1). Conversely, colony counts of Trichoderma increased greatly each season following treatment with allyl alcohol (Fig. 1.).

There was an increase in the number of Trichoderma colonies by the seventh day after treatment, and this condition held for the 8-week period of each seedbed season. Six months after the fifth crop was harvested, a final set of soil samples was taken from the plots for microbiological assay. Trichoderma levels continued to be high in the plots treated with allyl alcohol, whereas the population index of other fungi usually inhabiting the soil continued below normal. This is in sharp contrast to plots treated with other materials, such as methyl bromide (MC-2); in such plots, the initial sharp reduction in the general fungus population was temporary. Several days after the soil had been treated with MC-2, the dilution plates prepared from such soil were generally devoid of colonies; however, results of subsequent platings indicated that all the genera common to the location begin to reappear almost simultaneously thereafter. No above-normal population of *Trichoderma* spp. has been observed in soil treated with MC-2.

Discussion.—High populations of Trichoderma in plots treated with allyl alcohol may account for the observed superior effectiveness of the chemical in the control of damping-off organisms. The antagonism of *Trichoderma lignorum* (Tode) Harz against *Rhizoctonia solani* Kühn and species of *Pythium* has already been established by Weindling (16, 17), by Allen and Haenseler (1), and by Gregory et al (13). They showed clearly that Trichoderma interferes with the normal activities of these pathogens. Weindling and Emerson (18) found that all tested isolates of *T. lignorum*, of *T. koningi* Oud., and of *T. album* Preuss attacked the Rhizoctonia and that *T. lignorum* is capable of parasitizing *Rhizoctonia bataticola* (Taub.) Butl. and *Armillaria mellea* (Vahl) Quel. Allen and Haenseler demonstrated that filtrates of 5-day-old cultures of *T. lignorum* contained a toxic principle that inhibited the growth of Rhizoctonia.

Substrate acidity influenced the stability of the lethal substance studied by Weindling (17). Under laboratory conditions, deterioration of the lethal principle was rapid above pH 4.5. Observations in the present seedbed trials involving 2 levels of soil pH may support these findings by Weindling (17). Better control of diseases caused by soil-inhabiting fungi was evident in soils of pH 4.8–5.3 than in soils of pH 5.5–6.5. At the higher pH, Trichoderma was not as abundant and plant response following application of allyl alcohol was more erratic.

The ability of *T. lignorum* to destroy various patho-

Table 1.—*Prevalence of Fusaria and Trichoderma in a sandy soil treated with allyl alcohol alone and in combination with ethylene dibromide*

Organism	Year	Season	Number of colonies/g of dry soil from plots treated as indicated			
			Check	Allyl alcohol[a]	Allyl alcohol + EDB[b]	LSD (0.05)
			×10³	×10³	×10³	×10³
Fusaria	1952	Spring	435	168	62	136
		Fall	478	214	197	190
	1953	Spring	564	146	116	92
		Fall	288	39	42	77
	1954	Spring	503	48	67	101
Trichoderma	1952	Spring	80	213	234	27
		Fall	46	205	266	51
	1953	Spring	53	266	284	33
		Fall	143	397	390	48
	1954	Spring	182	365	378	38

[a] Applied at the rate of 25 gal. per acre.
[b] Allyl alcohol applied at the rate of 25 gal. per acre and EDB (85 per cent ethylene dibromide) at a rate of 4.5 gal. per acre.

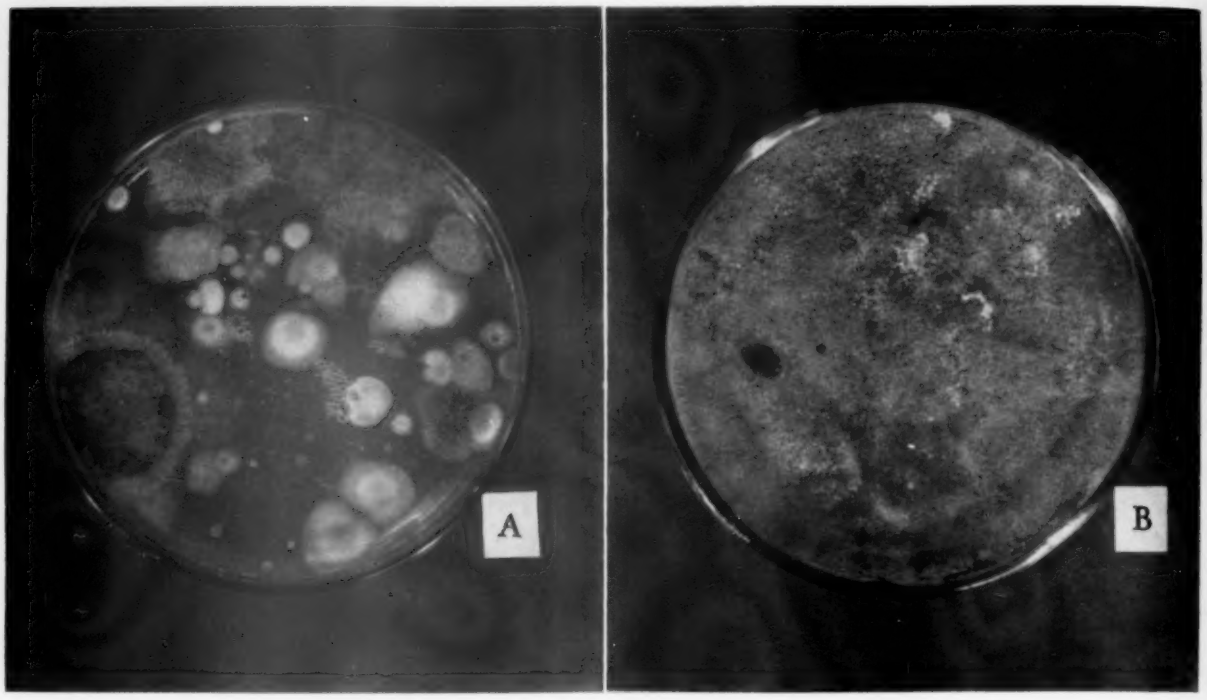


FIG. 1. Ten-day-old cultures on plates prepared from soil suspensions. A) From nontreated Leon fine sand, showing characteristic colonies of different fungi present in nontreated soil. B) From similar soil treated with allyl alcohol, showing the high incidence of *Trichoderma* species characteristic of treated soils.

gens introduced into otherwise sterile soil has been reported (17). *T. lignorum*, when introduced into sterilized soils artificially infested with *Helminthosporium sativum* Pam., King, & Bakke, suppressed seedling injury (10). By addition of *T. lignorum* to the seed as part of a pellet, Gregory et al (13) obtained seedling protection in soil that had been sterilized and then artificially infested with *Pythium debaryanum* Hesse.

The use of allyl alcohol may create conditions in the soil favorable for the development of Trichoderma. Also, allyl alcohol may be more toxic to other soil fungi than to Trichoderma, and these fungi may be directly eliminated from the soil. Fungi antagonistic to Trichoderma may be eliminated, permitting Trichoderma to develop unchecked. Undoubtedly, further information on several factors will be required before the most effective and practical procedures for its use can be determined. This has been demonstrated in microbiological tests of diverse soil types treated at different dosage rates. Twelve gal. per acre on Leon fine sand does not reduce the common soil fungi as effectively as does 25 gal. per acre, and the resulting population of Trichoderma is not as abundant. In contrast, there is evidence that 50 gal. per acre not only controls the harmful fungi but decreases the activity of the Trichoderma also.

Though allyl alcohol has controlled damping-off organisms and favored the establishment of a high population of Trichoderma in seedbed soils during the varying climatic conditions encountered in 5 crop seasons, much remains obscure concerning the relationship of the chemical to the presence and activity of *Trichoderma* spp.

GULF COAST EXPERIMENT STATION
BRADENTON, FLORIDA

LITERATURE CITED

1. ALLEN, M. C., AND C. N. HAENSELER. 1935. Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. Phytopathology **25**: 244-252.
2. BENNETT, R. R., AND S. N. HAWKS. 1951. Burley tobacco plant production guide. N. C. Agr. Col. Ext. Cir. 364, 16 p.
3. BURGIS, D. S. 1954. Production of vegetable plants in seedbeds on sandy soils. Fla. Agr. Exp. Sta. Bul. 550, 24 p.
4. BURGIS, D. S., AND A. JACK. 1952. Factors affecting germination of seed and growth of vegetable plants in seedbeds on sandy soil. Fla. Agr. Exp. Sta. Ann. Rpt. **1952**: 253-255.
5. BURGIS, D. S., AND A. JACK. 1953. Factors affecting germination of seed and growth of vegetable plants in seedbeds on sandy soil. Fla. Agr. Exp. Sta. Ann. Rpt. **1953**: 286-290.
6. BURGIS, D. S., AND A. J. OVERMAN. 1954. Factors affecting germination of seed and growth of vegetable plants in seedbeds on sandy soil. Fla. Agr. Expt. Sta. Ann. Rpt. **1954**: 261-262.
7. BURGIS, D. S., AND E. L. SPENCER. 1950. Seedbed studies. Fla. Agr. Exp. Sta. Ann. Rpt. **1950**: 134-135.
8. BURGIS, D. S., E. L. SPENCER, AND A. JACK. 1951. Seedbed studies. Fla. Agr. Exp. Sta. Ann. Rpt.

1951: 225-227.
9. CHAPPELL, W. E., AND J. L. LAPRADE. 1955. Weed control—tobacco seedbeds. South. Weed Conf. Proc., p. 213-216.
10. CHRISTENSEN, J. J. 1936. Association of microorganisms in relation to seedling injury arising from infected seed. Phytopathology **26**: 1091-1105.
11. EMMERT, E. M., AND J. E. KLINKER. 1950. Comparative use of aero cyanamid and allyl alcohol for controlling weeds in snapbeans and sweet corn. Ky. Agr. Exp. Sta. Prog. Rpt., 11 p.
12. FRED, E. B., AND S. A. WAKSMAN. 1928. Laboratory manual of general microbiology. McGraw-Hill, New York. 145 p.
13. GREGORY, K. F., O. N. ALLEN, A. J. RIKER, AND W. H. PETERSON. 1952. Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa. Phytopathology **42**: 613-622.
14. LINDGREN, R. M., AND B. W. HENRY. 1949. Promising treatments for controlling root disease and weeds in a southern pine nursery. U. S. Dept. Agr. Pl. Dis. Rptr. **33**: 228-231.
15. OVERMAN, A. J., AND D. S. BURGIS. 1956. Allyl alcohol as a soil fungicide. (Abs.) Phytopathology **46**: 22.
16. WEINDLING, R. 1932. Trichoderma lignorum as a parasite of other soil fungi. Phytopathology **22**: 837-845.
17. WEINDLING, R. 1934. Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. Phytopathology **24**: 1153-1179.
18. WEINDLING, R., AND O. H. EMERSON. 1936. The isolation of a toxic substance from the culture filtrate of Trichoderma. Phytopathology **26**: 1068-1070.

A DECLINE OF SOUR CHERRY CAUSED BY A VIRUS OF THE LITTLE CHERRY TYPE [1]

J. A. Milbrath and H. E. Williams

SUMMARY

A virus disease that causes a serious decline in growth of Montmorency sour cherry trees and small orange-pink fruits was first observed in 1951. The virus was transmitted by graft techniques to the Montmorency variety of sour cherry and to Bing, Lambert, Napoleon, and Black Republican varieties of sweet cherry. The outstanding symptoms of the disease on sour cherry are a marked decline in growth, small leaves, and small late-maturing fruit that are pink to orange in color. Infected sweet cherry foliage showed only a mild nutritional type of chlorosis, and a mild leaf rolling and some bronzing of the leaves as autumn approached. The sweet cherry fruits are small and late maturing. At normal maturity time, they are light in color but later become dark except for 2 bilateral depressed areas near the stylar end of the fruit on the side opposite the suture. The virus causes symptoms in sweet and sour cherry that are similar to those caused by the virus recently reported as being present in normal-appearing Kwanzan and Shirofugen flowering cherry.

A previously undescribed disease of sour cherry (*Prunus cerasus* L.) was observed in Polk County, Oregon, in 1951 in an orchard where the owner had been aware of a diseased condition in some of his trees for at least 6 years. He had attempted to control the diseased condition in 2 of the first trees infected by treating with various fertilizers, by pruning severely, and eventually by top working the trees with normal scions; these did not control the disease. By 1951, all trees in a large circular area of about an acre surrounding these 2 trees had become infected, and smaller areas had developed in other parts of the orchard. The Oregon State Department of Agriculture conducted surveys of the sour cherry orchards of the area in 1954 and 1955 and found the disease to be present in 11 of 22 orchards inspected.[2] The disease has not been found in cherry orchards in other parts of the state; however the nature of the symptoms on either sweet or sour cherry would make casual detection unlikely.

SYMPTOMS OF THE DISEASE ON SOUR CHERRY ORCHARD TREES.—In orchards the disease occurs in circular localized areas in which the most severely affected trees are in the center and lesser affected trees around the perimeter. Observations indicate that the more severely affected trees represent initial infections and that the lesser affected trees represent spread from these. Trees in advanced stages are sparsely foliated, with small leaves and little or no terminal growth. Severely diseased trees are easily detected, but in the early stages diseased trees are difficult to distinguish from normal trees. Likewise, the fruits on diseased trees show various degrees of injury. Trees showing severe foliage symptoms produce only small orange-pink fruits (Fig. 1) of little or no commercial value, whereas fruit on trees in the early stages of the disease may be fairly normal in size but slow to ripen, making uniform picking difficult without serious cullage. Lack of specific diagnostic symptoms on both foliage and fruit makes accurate field diagnosis

[1] Accepted for publication May 2, 1956.
Technical Paper No. 961, Oregon Agricultural Experiment Station.
[2] Heinis, J. L. 1955. Cherry viruses in Oregon in 1954 and 1955. Ore. Hort. Soc. 47th Ann. Rpt. (In press).

of early infections difficult. The rootstock appears to produce no differential effect, since affected Montmorency were found on both mazzard (*Prunus avium* L.) and mahaleb (*P. mahaleb* L.) rootstocks with apparently identical symptoms.

TRANSMISSION TO EXPERIMENTAL TREES.—*Sour cherry.*—Fourteen fruiting-age Montmorency sour cherry trees, 8 on mazzard roots and 6 on mahaleb, were inoculated by means of buds or scions from naturally infected trees showing sour cherry decline. All of the inoculated trees but 2 developed foliage, growth, and fruit symptoms typical of the disease. Branches near the inoculation points showed mild symptoms the year after inoculation, but general infection could not be detected until the second or third year. Trees on mazzard and those on mahaleb rootstocks reacted in much the same manner.

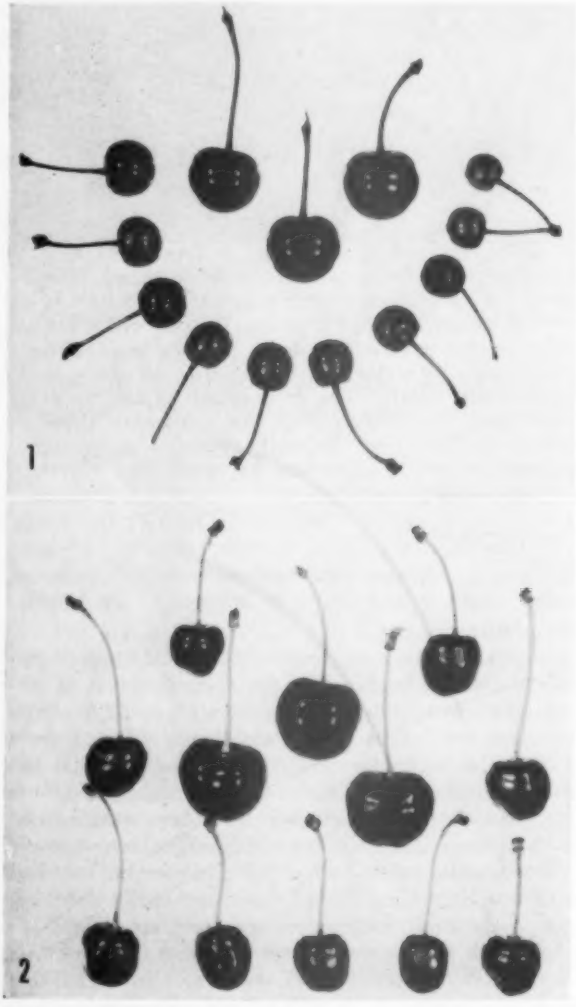


FIG. 1–2. FIG. 1. Three normal Montmorency sour cherry fruits surrounded by small orange-pink fruits from a naturally infected sour cherry tree that had symptoms of decline.—FIG. 2. Fruits from Bing sweet cherry; the 3 center fruits are from a normal tree and the others from a tree affected with sour cherry decline. The diseased fruits have developed normal color but are small and irregular in shape. (Photographs by H. H. Millsap.)

Sweet cherry.—Sixteen fruiting-age trees of sweet cherry (*P. avium*)—7 of the variety Bing, 3 of Napoleon, 4 of Black Republican, and 2 of Lambert—were inoculated by means of buds or scions from naturally infected Montmorency trees. In the year following fall inoculations, all of the trees except 1 Napoleon and 1 Black Republican developed fruit symptoms on inoculated branches and to some extent on adjacent branches. Dormant trees inoculated in the spring by means of terminal grafts of infected scions did not show fruit symptoms until the second growing season. By the second year after inoculation by either method, affected fruits were found on most branches of inoculated trees.

Fruit symptoms became apparent about 10 days before the fruits were ripe. As the normal cherries began to show rich red to black color, the more severely affected fruits remained smaller in size and much lighter in color (Fig. 2). On Bing, Lambert, and Black Republican trees, affected fruits eventually became as dark as normal fruits, except for 2 bilateral depressed areas that developed on some fruits opposite the suture near the stylar end. The skin and flesh in these areas were red in color, the skin became loose and slightly wrinkled, and the flesh was sunken. Many Lambert fruit and some Bing fruit fell while still light red in color. Color differences were more difficult to detect on Napoleon fruit, but severely affected fruits were smaller and developed less red blush than did normal fruits. The fruit also showed the 2 depressed areas.

When sweet cherry trees first became affected, only a portion of the fruits showed severe reduction in size; other fruits were normal in size and color, but the depressed areas opposite the suture caused a flattened condition on that part of the fruit. During the 3 years that the diseased trees have been studied, fruit on these have shown little if any tendency to be normal in size or shape.

Peach.—Only 2 trees of the J. H. Hale variety of peach (*Prunus persica* (L.) Batsch) have been inoculated directly from sour cherry trees showing decline; however, 19 trees of Hale, 3 of Rochester, 1 of Elberta, and 1 of Orange Cling, as well as 3 Lovell seedlings, have been inoculated from sour and sweet cherry trees that had developed typical symptoms following inoculation with the virus that causes sour cherry decline. None of these trees have produced symptoms that could be interpreted as due to any virus other than the complex of latent viruses commonly found in sweet and sour cherry. Return inoculations from the inoculated peach trees to fruiting-aged cherry trees have not been made.

RECOVERY FROM VARIOUS OTHER SOURCES OF THE VIRUS CAUSING SOUR CHERRY DECLINE.—During the last 10 years of stone fruit virus research at Oregon State College, a virus of the little cherry type, similar to the one causing the decline of sour cherry but different

from western X little cherry virus, has been recovered from several other source trees. The recovery of the virus from these trees was not a planned experiment, but resulted from contaminations in other virus cultures being used in various experiments. Considerable evidence also has been obtained to indicate that the recently reported little cherry type virus found in normal-appearing Kwanzan and Shiro-fugen flowering cherry trees[3] is the same virus as the one causing the decline of sour cherries.

The first such recovery of the virus was in 1944 in Lane County, Oregon, from a tree with symptoms typical of those caused by pink fruit virus.[4] Buds from this tree were used for the inoculations of 12 trees of Montmorency, 5 of Bing, 3 of Lambert, 4 of Napoleon, and 3 of Black Republican. Four of the Montmorency trees developed typical pink fruit symptoms and the rest remained normal. The inoculated sweet cherry trees developed a little-cherry condition that was assumed to be caused by the pink fruit virus. When return transmissions were made from the sweet cherry trees to normal Montmorency, the latter developed tree symptoms that were not characteristic for pink fruit but were similar to symptoms of trees inoculated by means of buds from sour cherry decline trees. Apparently this little cherry virus was carried as a contaminant in the original pink fruit tree.

Shiro-fugen has been used as a screening host plant in attempts to separate peach ring spot virus,[5] which is carried as a latent virus in most stone fruit trees, from viruses causing rusty mottle and sour cherry yellows. In 1 experiment involving the separation of peach ring spot virus and rusty mottle virus, 3 of the 5 Bing trees inoculated by means of Shiro-fugen buds developed little-cherry-type fruit symptoms. One of 2 other Bing trees inoculated by means of buds from a noninoculated Shiro-fugen tree developed the same little-cherry-type symptoms. Two of 3 Montmorency trees inoculated by means of buds from a Shiro-fugen used as a screening host for peach ring spot and sour cherry yellows viruses developed leaf and fruit symptoms of sour cherry decline. Two other Bing trees developed little-cherry-type symptoms when inoculated by means of buds from a dormant Napoleon with a dead bud condition. This tree was from Polk County where sour cherry decline was first observed. A little cherry condition occurred when a Lambert tree was inoculated by means of buds from a Black Republican tree. This tree had been purchased from a Willamette Valley nursery, and when it reached fruiting age the fruit was small and ripened unevenly. Two noninoculated Bing trees became naturally infected with the little-cherry-type virus in a row of experimental trees adjacent to some Montmorency trees inoculated by means of buds from trees showing sour cherry decline.

Three Lambert trees were inoculated by the use of 10 buds per tree from 3 normal-appearing Kwanzan trees. The little-cherry-type fruit symptoms that developed on these Lambert trees could not be distinguished from those on trees inoculated by means of buds from Montmorency with sour cherry decline or from those on any of the other little-cherry-affected trees discussed in the preceding paragraphs.

Discussion.—The virus discussed in this paper belongs to the little cherry group of stone fruit viruses.[6] The disease produced can be distinguished readily from western X little cherry, buckskin, albino, and small bitter cherry on the basis of fruit symptoms, virus behavior in the host, foliage symptoms, and the effect of mazzard and mahaleb rootstocks. The disease is very similar to little cherry as described for Kootenay, British Columbia,[6] and the exact relationship between the 2 diseases will have to wait a direct comparison under identical growing conditions. The 2 bilateral depressions on infected fruit and the persistence of the little-cherry-type fruit symptoms, especially on the Bing variety of sweet cherry, suggests that the virus discussed herein is a distinct virus strain. No technical name should be assigned to this virus until the relationship to the Kootenay little cherry disease can be adequately determined. "Sour cherry decline" has been used locally as the common name for the disease.

The little cherry disease described is well established in several western Oregon sour cherry plantings. Kwanzan and Shiro-fugen flowering cherries have been widely planted; if they are generally infected, the virus is worldwide in distribution. Three very important problems that should be investigated as soon as possible are apparent. Probably the most important problem will be to determine how general the infection is in Kwanzan, Shiro-fugen, and other flowering cherries. The virus possibly is too widespread to make eradication possible; therefore, as a second problem, a search for the vector of the virus should be made in those areas where the disease has been demonstrated to be spreading. Third, a quick and accurate diagnostic host will be needed as a tool for research work. The 2 or more years necessary for development of diagnostic symptoms on Montmorency is too long a period and the results are too indefinite for this purpose. The use of fruiting-age sweet cherry trees also is accompanied by many undesirable features. If some variety or species could be found that would give a good diagnostic leaf symptom, such a plant would be very valuable.

Department of Botany and Plant Pathology
Oregon State College
Corvallis, Oregon

[3] Reeves, E. L., P. W. Cheney, and J. A. Milbrath. 1955. Normal-appearing Kwanzan and Shiro-fugen oriental flowering cherries found to carry a virus of little cherry type. U. S. Dept. Agr. Pl. Dis. Rptr. 39: 725-726.

[4] Reeves, E. L. 1943. Virus diseases of fruit trees in Washington. Wash. State Dept. Agr. Bul. 1, 25 p.

[5] Milbrath, J. A. 1951. Separation of rusty mottle of cherry from a ring spot-rusty mottle complex. Science 113: 606-607.

[6] U. S. Dept. Agr. 1951. Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. of Agr. Agr. Handbook 10, 276 p.

TRANSMISSION OF FACTORS FOR UREDIOSPORE COLOR IN PUCCINIA GRAMINIS VAR. TRITICI BY MEANS OF NUCLEAR EXCHANGE BETWEEN VEGETATIVE HYPHAE[1]

R. R. Nelson[2]

SUMMARY

Puccinia graminis var. *tritici* was studied to determine whether factors for urediospore color could be transmitted by means of hyphal fusions and exchange of nuclei between vegetative hyphae. One of the mixtures of urediospores of red-brown race 11 and gray-brown race 121 resulted in the production of an orange biotype virulent on Vernal. After 6 uredial generations, all of the isolates of the orange biotype had produced red-brown and/or gray-brown dissociants. The gray-brown dissociants were identical in pathogenicity with the original gray-brown race 121. Thirty red-brown dissociants studied were intermediate in virulence between the original biotypes, and 2 red-brown dissociants exceeded both original biotypes in virulence. Orange isolates eventually were obtained which did not dissociate further.

Less than 1 per cent of the urediospores of the orange isolates was trinucleate. The orange isolates continued to produce some trinucleate spores as long as the isolate produced colored dissociants. The original biotypes and the red-brown and gray-brown dissociants had only binucleate urediospores and did not dissociate.

Recently it was reported[3] that hyphal fusions between races of *P. graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Guyot in the uredial stage produced new associations of nuclei and hence new races, some of which were more virulent than the original "parental" races. The principal objective of the research here reported was to determine whether factors for urediospore color could be transferred between races of *P. graminis* var. *tritici* by means of exchange of nuclei between vegetative hyphae.

Urediospore color in *P. graminis* var. *tritici* is determined by 2 independent factors, 1 for spore wall color and 1 for cytoplasm color, each of which is inherited in a 3:1 ratio[4]. Urediospores appear a normal red-brown when both factors are present in the dominant condition and colorless when both factors are homozygous recessive. When the factor for spore wall color is in the dominant condition, urediospores in mass have a gray-brown appearance when the factor for color of cytoplasm is homozygous recessive. Urediospores in mass appear orange when the factor for color of cytoplasm is dominant and the factor for spore wall color is homozygous recessive.

MATERIALS AND METHODS.—The basic techniques described in detail previously[3] were used in the present studies. Urediospores of a biotype of red-brown race 11 and of gray-brown race 121 of *P. graminis* var. *tritici* were mixed manually in approximately equal amounts and then diluted 1:5 with talcum powder.

The inoculum of each mixture was dusted on Little Club wheat (*Triticum compactum* Host), susceptible to both races, and Vernal emmer (*Triticum dicoccum* Schrank) resistant to race 11 and susceptible to race 121. Vernal emmer was used as the indicator wheat since no biotypes with colorless or orange urediospores used in these studies were pathogenic on that variety.

Measures were taken to assure the purity of cultures used. The purity of each race in a mixture was verified repeatedly on differential varieties.[5] Urediospores of each race to be included in a mixture were collected from plants of Little Club that were inoculated together with the other differential varieties. Little Club and Vernal were inoculated with both original biotypes separately at the same time that a mixture of urediospores was studied. The same isolates of both of the "parental" biotypes were used in all of the mixtures. Both "parental" biotypes were stable during all of the studies.

The production of different colored biotypes was detected directly on Vernal as a result of the initial inoculation. The first generation of urediospores produced on Little Club was transferred to Vernal and the resulting infections observed also for the production of different colored biotypes.

EXPERIMENTAL RESULTS. — *Greenhouse studies.* — Twelve mixtures of urediospores of red-brown race 11 and gray-brown race 121 were studied on a total of approximately 80,000 plants. One of the mixtures of urediospores resulted in the production of 1 orange pustule on Vernal. The orange pustule was increased

[1] Accepted for publication May 2, 1956.
Paper No. 3518, Scientific Journal Series, Minnesota Agricultural Experiment Station.
Supported in part by the Rockefeller Foundation.

[2] Formerly Research Fellow, University of Minnesota; now Agent (Pathologist), U. S. Department of Agriculture, Raleigh, North Carolina.

[3] Nelson, R. R., R. D. Wilcoxson, and J. J. Christensen. 1955. Heterocaryosis as a basis for variation in Puccinia graminis var. tritici. Phytopathology 45: 639-643.

[4] Newton, Margaret, T. Johnson, and A. M. Brown. 1930. A study of the inheritance of spore color and pathogenicity in crosses between physiologic forms of Puccinia graminis tritici. Sci. Agr. 10: 775-798.

[5] Stakman, E. C., M. N. Levine, and W. Q. Loegering. 1944. Identification of physiologic races of Puccinia graminis tritici. U.S. Dept. Agr. Bur. Ent. and Plant Quar. Bul. 617.

TABLE 1.—*Reaction of the differential varieties of Triticum spp. to race 11, to race 121, and to the orange biotype (red 11 + gray 121) of Puccinia graminis var. tritici*

Differential varieties	Race and varietal reaction[a]		
	11	121	Orange biotype
Marquis	S	S	S
Reliance	S	R	S
Kota	S	R	S
Lee	R	R	R
Arnautka	S	R	R
Mindum	S	R	R
Spelmar	S	R	R
Kubanka	S	S	S
Acme	S	S	R
Einkorn	S	S	S
Vernal	R	S	S
Khapli	R	R	R

[a] S indicates variety was susceptible; R indicates variety was resistant.

on Little Club, and 6 single-uredium isolates then were obtained from Little Club. These were passed through 3 uredial generations on Vernal and Little Club with no deviation from the orange urediospore color. The varieties of the differential series gave the same reactions to all of the 6 isolates (Table 1). The virulence of the isolates did not correspond to that of any known physiologic race of *P. graminis* var. *tritici*.

The first indication of dissociation of the orange biotype was observed during the fourth uredial generation. In this generation, 2 of the orange isolates produced some red-brown uredia on Little Club and on Vernal (Table 2). Then in the fifth uredial generation, 4 of the orange isolates produced red-brown uredia and a fifth isolate produced both red-brown and gray-brown uredia on Little Club and Vernal. All 6 of the orange isolates produced red-brown and/or gray-brown dissociants by the sixth uredial generation.

When all of the orange isolates had produced colored dissociants, investigations were made to determine whether orange biotypes could be obtained that would continue to produce only orange colored uredia. During the seventh uredial generation, single orange uredia of each of the 6 orange isolates were isolated and observed for the production of red or gray dissociants. If such dissociants were produced during the next generation, another orange uredium was isolated and increased and observed again for the production of colored dissociants. This technique was repeated until orange isolates eventually were obtained that produced only orange uredia through 4 additional generations.

TABLE 2.—*Dissociation of isolates of the orange biotype (red 11 + gray 121) during the first 7 uredial generations*

Isolate	Spore color[a] in indicated uredial generation				
	2-3	4	5	6	7
1	O	OR	OR	ORG	ORG
2	O	O	OR	OR	ORG
3	O	O	OR	ORG	ORG
4	O	OR	ORG	ORG	ORG
5	O	O	OR	OR	OR
6	O	O	O	OR	OR

[a] O, orange; R, red; G, gray.

Thirty-two isolates of red-brown dissociants and 4 isolates of gray-brown dissociants were increased as individual isolates on Little Club and tested on the differential series. All of the gray-brown isolates were identical in pathogenicity to the original gray-brown race 121. In pathogenicity, 30 of the 32 red-brown isolates were intermediate between the "parental" races 11 and 121; none were pathogenic on Vernal. Two of the red-brown isolates were more virulent than either race 11 or race 121 in that the 2 isolates attacked all of the differential varieties except Lee and Khapli and thus were similar to physiologic race 15. The original red-brown race 11 was not recovered from any of the isolates. None of the red-brown isolates or of the gray-brown isolates dissociated further into other colored biotypes.

Cytological studies.—The cytological materials and techniques were similar to those described earlier[3]. Leaf tissues, containing 1 or more pustules, were killed and fixed in weak chrom-acetic solution, dehydrated in normal butyl alcohol, and embedded in paraffin. Flemming's triple stain and the quadruple stain were used to stain nuclei in urediospores and in associated hyphal cells.

The urediospores and hyphal cells of the original races, red-brown 11 and gray-brown 121, were binucleate. All of the urediospores and hyphal cells of the red-brown dissociants and gray-brown dissociants that were observed were binucleate. No deviation from the binucleate condition was noted.

Urediospores of the 6 original orange isolates were examined during the uredial generations in which the isolates were dissociating into red-brown and gray-brown. Less than 1 per cent of the urediospores were trinucleate, and the remainder were binucleate. There was considerable variation among single pustules of the orange isolates. Some of the pustules examined had only binucleate urediospores; in others, from a small fraction of 1 per cent to nearly 1 per cent of the spores examined were trinucleate. No cytological studies were made of the orange isolates before they had begun to dissociate.

As long as orange isolates continued to produce red-brown and gray-brown dissociants, trinucleate urediospores could be found; however, when orange isolates had become stabilized for the production of orange uredia, all urediospores examined were binucleate.

DISCUSSION.—The results of this research present additional evidence that hyphal fusions and subsequent transfer and regrouping of nuclei between biotypes of *P. graminis* var. *tritici* result in the production of biotypes genetically different from the original parent biotypes. The results also present added evidence that the binucleate condition in urediospores is relatively inflexible and that multinucleate cells invariably tend to revert to the dicaryophase, although such reversion

may require several generations and may result in the production of biotypes genetically different from the original biotypes.

The cytological studies indicate that 1 of at least 2 nuclear conditions of the urediospores could have existed in the original orange pustule that was produced on Vernal. Possibly all of the urediospores in the original orange pustule were trinucleate; as the biotype dissociated, the binucleate dissociants resulted in the production of orange, gray-brown, or red-brown biotypes, dependent upon which of the 3 nuclei was lost through the dissociation. Second, some of the urediospores in the original orange pustule may have been binucleate and others trinucleate, both nuclear conditions resulting in the production of orange urediospores. As the trinucleate urediospores dissociated, orange, gray-brown, or red-brown biotypes could have been produced, again dependent on which of the 3 nuclei was lost.

The production of the orange heterocaryon on Vernal emmer demonstrates that hyphal fusions and passage of nuclei may occur on a host plant that is not susceptible to both original biotypes.

The fact that both a trinucleate condition and a binucleate condition apparently resulted in the production of orange urediospores suggests that the genic response of a heterocaryon may not necessarily be governed by the presence of an added gene (either for spore wall color or for color of cytoplasm), but might be due to the association of 2 nuclei needed to express orange color. It also projects the possibility that the Khapli heterocaryon, discussed previously,[3] may have been acting as a dicaryon on genic action and that the subsequent dissociation of the virulent biotype resulted in the loss of 1 of the nuclei required to express or maintain virulence.

The production of a dicaryotic dissociant more virulent than either original biotype demonstrates again that the genes for increased virulence on a given host plant may be expressed when placed in proper combination.

Department of Plant Pathology and Botany
Institute of Agriculture
University of Minnesota
St. Paul 1, Minnesota

CROSS PROTECTION WITH TWO RUST FUNGI[1]

C. E. Yarwood

SUMMARY

When adequate dosages of uredospores of *Uromyces phaseoli* were placed on sunflower leaves before inoculation of the leaves with *Puccinia helianthi*, or along with the inoculum, the sunflower leaves were protected from infection with *P. helianthi*. Similarly, spores of *P. helianthi* protected bean leaves from infection with *U. phaseoli*.

To give 50 per cent protection of sunflower from *P. helianthi* under the conditions of these tests required about 1.3 mg of uredospores of *U.phaseoli* per square decimeter of sunflower leaf surface. Under the same experimental conditions and on the basis of some inadequately confirmed assumptions, it is calculated that the inhibitor in the spores of these fungi has at least 10 times the fungicidal activity of Bordeaux mixture and half the fungicidal activity of zineb for these fungi.

Acquired immunity and cross protection are well-established phenomena with several virus infections of plants (2) but not with the infections of plants caused by any other type of pathogen. Preliminary evidence (9) has indicated acquired immunity and cross protection with the bean rust, broad bean rust, and snapdragon rust pathogens. The basic phenomenon of of self-inhibition of uredospores of rust fungi has been confirmed with the wheat stem rust fungus by Allen (9) and Forsyth (3). The present report is of cross protection with the bean rust, sunflower rust, and snapdragon rust fungi. The methods used were different from, and more quantitative than, methods used previously.

Materials and methods.—Inoculations with water suspensions of uredospores of the sunflower rust fungus (*Puccinia helianthi* Schw.) were made on the first true leaves of sunflower (*Helianthus annuus* L.) plants at about 15 days from seeding in 4-in. pots in the greenhouse. Similarly, inoculations with the bean rust fungus (*Uromyces phaseoli* (Pers.) Wint.) were made on the primary leaves of Pinto bean (*Phaseolus vulgaris* L.) at about 11 days from seeding. Neither of these pathogens attacked the host of the other under the conditions of these tests, except as otherwise mentioned.

[1] Accepted for publication May 23, 1956.

To test the protective action of the bean rust fungus against the sunflower rust fungus in preliminary trials, unmeasured dosages of dry uredospores of the former were dusted onto the lower surfaces of sunflower leaves and the leaves then inoculated with water suspensions of uredospores of the sunflower pathogen. The protection resulting was so striking that quantitative methods were developed. In the quantitative tests, sunflower leaves were inoculated with a series of spore suspensions in which the concentration of uredospores of the sunflower rust fungus was held constant and the amounts of uredospores of the bean rust pathogen were varied from none to large dosages. When increasing dosages of the latter caused decreasing amounts of infection by the former, cross protection is assumed to have resulted.

The large, simple, petiolate, and opposite leaves of each host are considered very advantageous for these studies, and twin leaf methods with attached leaves were used throughout. For example, one sunflower leaf of each pair was inoculated with the control suspension of uredospores of *P. helianthi* only, whereas the opposite leaf was inoculated with the same concentration of such uredospores plus a measured concentration of uredospores of *U. phaseoli*. The spore suspensions were prepared a few minutes before inoculation by washing the loose mature spores from living heavily infected leaves by means of a water spray.

Uredospores of rust fungi are oily in behavior; they are difficult to wet and to bring into uniform suspension; they tend to remain in clumps, and many remain floating on the surface of the water. Most uredospores can be brought into fairly uniform suspension by strong agitation in water, and this method was commonly used but is not completely satisfactory.

The dosage of spores on inoculated leaves was manipulated and determined by 3 methods. In one, spores were shaken from dry leaves of heavily infected plants into a large beaker, and weighed amounts of dry spores were added to known amounts of water to give a known concentration of spore suspension. This was sprayed onto the lower leaf surfaces of test plants in a manner that gave medium deposit without runoff. This deposit was about 0.8 g of wet spray deposit per square decimeter of leaf surface. From this, the spore deposit, as weight of spores per unit area, could be calculated. For example, a 0.1 per cent suspension of spores would give a spore deposit of about 0.8 mg/dcm^2.

With another method, suspensions containing unmeasured amounts of spores were used as inoculum, and the number of spores per unit area of inoculated leaves was counted directly on the surface-dried leaves with the aid of Zeiss surface illuminating objectives. From this and from the known fresh or dry weight per spore, the deposit as mg/dcm^2 could be calculated. Unfortunately, spore deposit as measured in this way was very uneven. The average deviation of a single determination of spores on 0.47 mm^2 areas of leaf surface from a mean of 27 determinations on the same treatment was 67 per cent of the mean when the mean number of spores was 5.2 spores per 0.47 mm^2 in 1 representative test.

By the third method, spore deposits were calculated from the numbers of spores per unit volume of suspension and the known unit deposit. To measure the number of spores in suspension, 0.01 per cent final concentration of Tween 60 (a polyoxyalkylene derivative of sorbitan monostearate) was added to the spore suspension as used. This was then diluted as needed with more 0.01 per cent Tween, 0.01-ml aliquots were deposited as drops on slides, and all the spores in each drop were counted. For example, if a suspension averaged 58 spores per 0.01 ml after a 1-1000 dilution, the original suspension contained 5.8×10^6 spores/ml and could be expected to deposit about 4.6×10^6 spores/dcm^2. This could be converted to weight of spores per unit area by multiplying by the weight per spore, to be given later.

In 3 trials, the inoculum averaged 15,600 spores per ml, and this deposited a measured average of 150 spores per cm^2. The result was an average of 35 pustules per cm^2. This indicated an average of about 23 per cent efficiency of inoculum, but in 1 trial there was 64 per cent efficiency.

To determine the fresh or dry weight of spore deposits per unit area of leaves by 2 of the methods given, the weight per spore must be known. Two methods of determination were used. In one, a weighed amount of spores was diluted with a relatively large amount of water and the number of spores in 0.01-ml aliquots was counted. From this number, from the total weight of spores, and from the volume in which they were suspended, the weight per spore can be calculated. For example, 0.205 g of spores of *P. helianthi* suspended in 10,000 ml of water gave 6010 spores per ml, and the weight per spore was 3.4×10^{-9} g ($= 3.4 \times 10^{-6}$ mg). By the other method, the calculated volume per spore was multiplied by the density. When observed dry in the microscope, uredospores of both rust fungi from infected leaves in the greenhouse have a doughnut-like shape, but with a depression in the center instead of a hole. Those of *P. helianthi* have a diameter of about 23 μ and an average thickness of about 13 μ. If the average diameter is then taken as 18 μ and the volume calculated as a sphere, the volume per spore is about 3100 μ^3. If the density of the spores is taken as 1.36 (7), the average fresh weight per spore would be 4.2×10^{-9} g. This is considered fair agreement with the previous method, and the average of 3.8×10^{-9} g fresh weight per spore will be used in the report. If 7.4 per cent is used as the water content of fresh spores (6), the dry matter content per spore is 3.5×10^{-9} g. The uredospores of *U. phaseoli* are only slightly different morphologically from those of *P. helianthi*, and in this study the same values will be used for each.

In addition to expressing results in terms of the amount of spores to bring about 50 per cent reduction in infection (ED_{50}), results are expressed as fungicide coefficients. Fungicide coefficients as used here are the ratios of the ED_{50} of standard fungicides, expressed as dry weight, to the ED_{50} of spores or of inhibitor in spores under comparable conditions. For example, if the ED_{50} of Bordeaux mixture were 2 mg and the ED_{50} of spores were 20 mg, the Bordeaux coefficient of spores would be 0.1. To calculate ED_{50} in terms of actual inhibitor, some assumptions must be made. We might assume that under temperature and humidity conditions favorable for germination, the total loss of dry weight of spores, other than the loss due to respiration, is inhibitor. Allen (1) has presented evidence that CO_2 of respiration is not involved in the self inhibition. Respiration, as CO_2 evolution, and dry weight loss of spores were measured in 1 trial with duplicate samples of about 100 mg spores each at 23°C for 29 hours. The initial water content of the spores was 5.6 per cent of their fresh weight. From 208 mg fresh weight of spores, 10.35 mg CO_2 was evolved by respiration and 13 mg dry weight was lost. This respiration corresponds to a dry weight loss of 7.1 mg or 3.4 per cent of the original fresh weight or 3.6 per cent of the original dry weight. Respiration, therefore, accounted for only 55 per per cent of the measured loss of dry weight. If we assume that the remaining 45 per cent of the loss was inhibitor, we have an inhibitor content of 3 per cent of the dry weight of the spores. This is a maximum value from the writer's data. The true value is probably somewhere between 3 per cent and 0 but cannot be determined from the present data. From Allen's data (1), however, the most nearly comparable figure is 7.5 per cent for the inhibitor content of spores. If we apply the value of 3 per cent inhibitor content to the known deposits of spores, we can calculate the protection value of the inhibitor.

In dosage response results from cross-protection trials, dosage is given as milligrams dry weight of spores per square decimeter of leaf surface (mg/dcm^2). This value can be converted to fresh weight of spores by multiplying by 1.08, to numbers of spores by multiplying by 2.6×10^5, and to calculated amount of inhibitor by dividing by 33.3.

RESULTS.—*Cross protection.*—Data for all trials of the protective value of uredospores of *U. phaseoli* against infection with *P. helianthi* in which spore dosage was measured are given in Figure 1, and a representative example is illustrated in Figure 2. The ED_{50} ranged from 0.46 to 3.5 mg spores/dcm^2 and averaged 1.3 mg. The slope of the dosage response curve expressed as probits control divided by logarithmic units of dosage was about 2.9. Other ED_{50} values determined in the same way were 0.4 mg/dcm^2 for Bordeaux (calculated as total dry matter) against *P. helianthi*, 0.02 mg/dcm^2 for zineb (zinc ethylenebis [dithiocarbamate]) against *P. helianthi*, 4 mg/dcm^2 for uredospores of *P. helianthi* against *U. phaseoli*

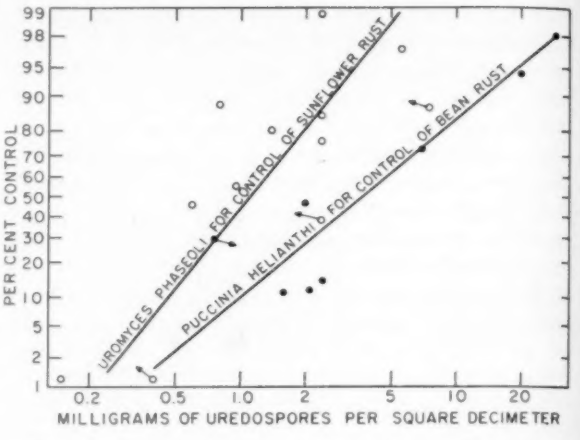


FIG. 1. Dosage response relations for uredospores of *Uromyces phaseoli* against sunflower rust and for uredospores of *Puccinia helianthi* against bean rust.

(Fig. 1), 0.3 mg/dcm^2 for Bordeaux against *U. phaseoli*, and 0.02 mg/dcm^2 for zineb against *U. phaseoli*. The Bordeaux and zineb coefficients for the protective value of dry spores of *U. phaseoli* against *P. helianthi* were, therefore, about 0.3 and 0.015, respectively. If we assume 3 per cent of the dry weight of the spores is inhibitor, then the Bordeaux and zineb coefficients of the inhibitor are about 10 and 0.5, respectively.

Self protection.—One might expect that inoculation of sunflower with high concentrations of uredospores of *P. helianthi* or inoculation of bean with high concentrations of uredospores of *U. phaseoli* would result in low infection (self protection) because of the self inhibition of spore germination that occurs *in vitro* (1, 3, 10). This was not the case. Infection increased with increased dosage of spores, although the number of pustules per spore decreased with increasing spore concentration. At the highest concentrations of spores tested (about 10 mg/dcm^2), heavy infection resulted and the pustules were so numerous and close together it was not possible to count them. Reasons for the failure of self protection under these conditions are not known. It is possible that self inhibition approaches 100 per cent asymptotically but is never complete. If 10 mg of spores were deposited per dcm^2 and only 1 per cent germinated and caused infection, there would still be 240 pustules per cm^2, which is far more than can be counted by the methods used. Self protection (also called acquired immunity) has been demonstrated, however, by other methods (9).

Cross infection.—Inoculation of sunflower with uredospores of *U. phaseoli* usually resulted in no macroscopic symptoms. In some trials, flecking resulted on the distal portions of certain leaves. On bean inoculated with *P. helianthi*, a transient but pronounced upcurling of the leaves (11) was seen on some plants that were removed from the incubation

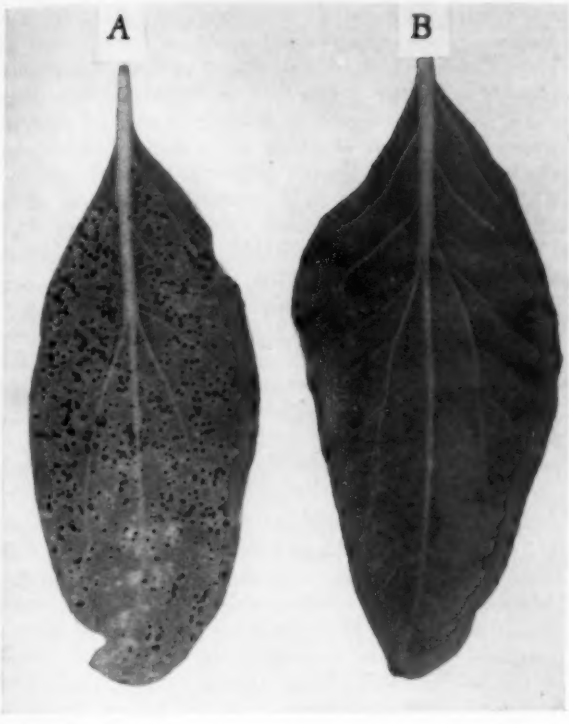


FIG. 2. Protective action of uredospores of *U. phaseoli* against sunflower rust. A) Control sunflower leaf with no protective deposit and with heavy infection with *P. helianthi*. B) Opposite leaf to A, but with protective deposit of uredospores of *U. phaseoli* and inoculated with same dosage of *P. helianthi* as A. The dark patches on B are concentrated deposits of uredospores of *U. phaseoli*.

moist chambers at about 15 hours after inoculation, but no pustule-like structures were observed.

Cross protection with strains of the same pathogen.—When beans or snapdragons (*Antirrhinum majus* L.) were first inoculated with a strain of their rust fungus to which they were immune and later inoculated with a strain to which they were highly susceptible, infection from the second inoculation was usually less than when the first inoculation was omitted. This method was designed to determine if the incipient infections by foreign rust fungi could protect a host from infection with a rust fungus to which it was susceptible, and it was used at first in ignorance of the later-discovered phenomenon of self inhibition of spore germination. Golden Gate Wax beans were inoculated with form 4 of *U. phaseoli*, to which they were immune[2]; these and control leaves were inoculated 1, 2, 3, 5, or 7 days later with form 2, to which they were susceptible. Similarly, a strain of Brown Kentucky Wonder beans was inoculated with form 2, to which it is immune, followed by inoculation with form 4, to which it is susceptible. In 4 trials, the amount of protection as a result of prior inoculation with a strain to which these varieties were immune ranged from 0 to 76 per cent and averaged 33 per cent. The dosage of spores for the first inoculation in these trials was not measured but was much lower than in the trials of cross protection between the bean and sunflower rust fungi.

Twenty trials were made to test the possible protective effect of form 1 of the snapdragon rust fungus (*Puccinia antirrhini* Diet. & Holm.) against form 2 (4), on the so-called rust-resistant snapdragons. Whereas some cross protection resulted in most trials, the results were erratic and the protection averaged less than with strains of *U. phaseoli* mentioned above.

Reinoculation following heat and pressure therapy.—Mycelium of *U. phaseoli* in living leaves can be killed by heat (5) or by pressure (8) with only slight host injury. Reinoculation of the areas in which the infection had been thus cured resulted in heavy infection under all conditions tested.

Miscellaneous observations.—In reports by Allen (1), Forsyth (3), and Yarwood (9), the volatile nature of the self inhibitor of rust fungi has been emphasized. Two bits of recent evidence might seem to depreciate the importance of volatility.

First, it was thought that since the inhibitor is volatile it should be possible to increase its potency by confining the gas during tests of acquired immunity. Opposite bean leaves, strips of which were already infected (9, Fig. 1), were reinoculated and allowed to dry in the greenhouse. One leaf of each pair was sealed between the outside surfaces of petri dish halves of equal diameter. This confined the gas from the infected parts of this leaf to a volume of about 15 ml. The opposite control leaf was not especially enclosed, and the entire plant was placed overnight in a moist chamber incubator with a volume of about 40,000 cc. In 1 trial in which this type of treatment was replicated twice, the average distance of total inhibition of the second infection from the first (see 9, Fig. 1) was about 5 mm for the confined leaves and 7 mm for the controls. The original hypothesis was thus not confirmed. If it is assumed that these distances of inhibition are equal, it might be concluded that the effective self inhibitor moved principally through the intercellular spaces of the leaf rather than as an external blanket of vapor. If it is concluded that the distance of inhibition was greater for the control leaves than for the confined leaves, this test gives emphasis to the adsorption of the inhibitor by glass, as found by Allen (1).

Secondly, it was thought that if the inhibitor were much more soluble in water than in air, it could be largely removed by soaking the leaves in water. Two bean leaves, strips of which were infected by *U. phaseoli*, were soaked in water in the greenhouse for 8 hours. The opposite control leaves, strips of which were similarly infected, were left exposed to air. No water infiltration of the intercellular spaces was observed. The water-soaked leaves were then surface-

[2] Dundas, B. J. 1940. Unpublished information. The varieties of bean and the strains of *U. phaseoli* for this part of the study were obtained from Mr. Dundas.

dried and all leaves were inoculated with *U. phaseoli*. Infection without any inhibition zone resulted on the soaked leaves, whereas on the control leaves an inhibition zone was clearly apparent. Although this could be explained by the much greater solubility of the inhibitor in water than in air, it could also be explained by suppression of formation of inhibitor in water-soaked leaves.

DISCUSSION.—Whereas cross protection with rust fungi is superficially similar to cross protection with viruses (2), the 2 types of cross protection are probably fundamentally different. Although that with plant viruses is better known and is represented by many more examples, it is hardly better understood now than when first discovered: it cannot generally be demonstrated *in vitro*, and no theories as to its basic nature seem well accepted. The chemical, if there be one, responsible for cross protection with viruses apparently is different for each virus or virus group, since cross protection may result between closely related viruses but not between distantly related ones.

Cross protection with rust fungi, as presently understood, may be relatively simple. Trimethylethylene (3), and perhaps other chemicals, is released by spores and mycelium of rust fungi and when present in adequate amounts will prevent spore germination. Since cross protection occurs between different genera (e.g. *Puccinia* and *Uromyces*) it appears that the same chemical is released by quite unrelated rust fungi. This property of these fungi may play an important role in their development in nature. In a mature uredinial pustule with thousands of spores, the spores do not germinate, or do so rarely, even if placed under favorable conditions of temperature and humidity. If the spores did germinate under these conditions, they would have little chance of causing a new infection or of propagating themselves because the host is already heavily parasitized in their vicinity. The phenomenon of self inhibition preserves them until they are disseminated and can germinate on tissues not already parasitized. If large numbers of spores per unit area fall on healthy leaves, the self inhibitor might permit only relatively few to infect. This also would favor the fungus, for if large numbers of infections occurred per unit area, say 200/cm^2, the total number of spores produced by the fungus would be much less than with 2 infections/cm^2 (supporting data available but not presented here). This same inhibitor apparently does not inhibit mycelial growth (9) and, therefore, does not reduce invasion after host penetration has succeeded.

These studies were partially to explore the possibility of control of rusts by means of the self inhibitor. In terms of chemical effectiveness, the results indicate that the inhibitor is better than Bordeaux mixture, a standard fungicide, though poorer than zineb, which is the best protective fungicide for bean rust tested by the writer. Whereas the use of spores as a protective coating is impractical, the use of purified inhibitor would seem to be worthy of further exploration.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

LITERATURE CITED

1. ALLEN, P. J. 1955. The role of a self-inhibitor in the germination of rust uredospores. Phytopathology **45**: 259-266.
2. BENNETT, C. W. 1953. Interactions between viruses and virus strains. In K. M. Smith and M. A. Lauffer, ed. Advances in virus research. v. 2. Academic Press, New York. p. 39-67.
3. FORSYTH, F. R. 1945. The nature of the inhibiting substance emitted by germinating urediospores of Puccinia graminis var. tritici. Canad. Jour. Bot. **33**: 363-373.
4. YARWOOD, C. E. 1937. Physiologic races of snapdragon rust. Phytopathology **27**: 113-115.
5. YARWOOD, C. E. 1948. Therapeutic treatments for rusts. Phytopathology **38**: 542-551.
6. YARWOOD, C. E. 1950. Water content of fungus spores. Amer. Jour. Bot. **37**: 636-639.
7. YARWOOD, C. E. 1952. Some water relations of Erysiphe polygoni conidia. Mycologia **44**: 506-522.
8. YARWOOD, C. E. 1953. Pressure effects in fungus and virus infections. Phytopathology **43**: 70-72.
9. YARWOOD, C. E. 1954. Mechanism of acquired immunity to a plant rust. Nat. Acad. Sci. Proc. **40**: 374-377.
10. YARWOOD, C. E. 1956. Simultaneous self-stimulation and self-inhibition of uredospore germination. Mycologia **48**: 20-24.
11. YARWOOD, C. E., AND M. COHEN. 1956. Hypertrophy from the uredial stage of bean rust. Bot. Gaz. **112**: 294-300.

BLACK SHANK RESISTANCE IN FLUE-CURED TOBACCO AS INFLUENCED BY TOLERANCE TO CERTAIN PARASITIC NEMATODES [1]

E. L. Moore, P. N. Drolsom, F. A. Todd, and E. E. Clayton [2]

SUMMARY

The rapid spread of black shank caused by *Phytophthora parasitica* var. *nicotianae* has caused many flue-cured-tobacco growers to change to resistant varieties. High-yielding varieties such as Dixie Bright 101 have been only moderately resistant. In North Carolina from 1951 to 1954, 20 to 25 per cent of the acreage was planted with Dixie Bright 101. During 1951, the first year of wide-scale planting with this variety, and in each succeeding year, it was severely diseased with black shank in a small percentage of interest fields. In these cases root-knot (*Meloidogyne* spp.), meadow (*Pratylenchus* spp.), and possibly stunt (*Tylenchorhynchus claytoni*) nematodes appeared to influence the development of black shank. To meet this situation, Dixie Bright 102, related to Dixie Bright 101 but more resistant to black shank, was crossed with Hicks and Bottom Special. The latter 2 are varieties of unknown origin which are well adapted in areas where the 3 named species of nematodes are prevalent. Fields where Dixie Bright 101 survived poorly were used as black shank testing nurseries. Three F_6 selections from the Dixie Bright 102 × Hicks cross were more resistant than Dixie Bright 102, and plants of one of these survived 5 times better than those of the resistant parent in 1954. An F_5 selection from Dixie Bright 101 × (Dixie Bright 102 × Bottom Special) was intermediate in resistance between the 2 resistant parents. Both families of selections developed in an apparently normal fashion while supporting moderate populations of meadow and root-knot nematodes in the presence or absence of black shank. These F_5 and F_6 lines produced 14–30 per cent higher yields and more top quality grades than Dixie Bright 101.

Tobacco black shank, caused by *Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker, was first identified on flue-cured tobacco in North Carolina in 1931 (13). Since then it has become one of the most widespread and destructive diseases of this crop. The most effective control measure is the use of resistant varieties in combination with crop rotation. The first resistant variety was released in 1943 (1), and since then several have been developed (7, 9, 12). In North Carolina tests, Dixie Bright 101 was the first of these to produce yields comparable to the commonly used susceptible varieties. Its high yields and moderate black shank resistance made it popular with farmers greatly in need of relief from the increasingly critical black shank problem (11). Resistance to bacterial wilt, caused by *Pseudomonas solanacearum* E. F. Sm., and Fusarium wilt, caused by *Fusarium oxysporum* (Schlecht.) var. *nicotianae* J. Johnson, added further to the utility of Dixie Bright 101. Two years after its release in 1949, an estimated 20–25 per cent of the tobacco acreage in North Carolina was planted to this variety. Dixie Bright 102, a low-yielding companion variety, never was planted extensively.

During the course of development of Dixie Bright 101 and Dixie Bright 102, tests were conducted in 4 nurseries in the eastern, middle, and western parts of that area in North Carolina where flue-cured tobacco is produced. In 12 black shank tests from 1948 to 1951, where 99.9 per cent of susceptible check plants were killed, only 18.4 per cent of Dixie Bright 101 plants and 8.7 per cent of Dixie Bright 102 plants showed black shank symptoms. During 1951, extensively planted Dixie Bright 101 suffered losses up to 95 per cent in certain fields infested with the black shank organism. In many cases, these high losses occurred where tobacco had been planted the previous year.

Crop rotation is a recommended practice to be used with resistant varieties to control parasitic nematodes and to reduce black shank severity (3, 5); however, many farmers have insufficient land suitable for tobacco culture to follow completely the recommended crop rotation practices. Although highest losses of Dixie Bright 101 occurred in fields planted to tobacco the previous year, significant losses followed other crops known to increase nematode populations (Fig. 1). Soil and root samples were taken in 15 such fields where Dixie Bright 101 plants were stunted and showed black shank symptoms. Root-knot nematodes (*Meloidogyne* spp.) were found in 11, meadow nematodes (*Pratylenchus* spp.) in 7, and stunt nematodes (*Tylenchorhynchus claytoni* Steiner) in 6 fields. Either root-knot or meadow nematodes were the only parasitic forms present in each of 3 fields, whereas stunt nematodes never were found alone. However, Graham (6) has shown that tobacco is damaged by stunt nematodes under certain conditions.

In addition to the presence of parasitic nematodes in certain fields, observations provided a further clue

[1] Accepted for publication May 16, 1956.

Contribution from Agricultural Research Service, U. S. Department of Agriculture, and Plant Pathology, North Carolina Agricultural Experiment Station; published with the approval of the Director of Research as Paper No. 735 of the Journal Series.

[2] Research Associate Professor of Plant Pathology, North Carolina State College, and Agronomist, U. S. Department of Agriculture; Pathologist; formerly Pathologist; and formerly Pathologist, respectively, Agricultural Research Service, U. S. Department of Agriculture.

to nematode complicity in problem areas. A commercial seed mixture of Dixie Bright 101 with Hicks, a highly popular variety susceptible to black shank, resulted in the interplanting of these 2 varieties in black-shank-free fields. In some fields there was disparity of growth between the 2 varieties. The Hicks

FIG. 1–2. Fig. 1. Effect of crop rotation on Dixie Bright 101. Left: following watermelons; 2-g samples of roots contained few meadow nematodes (*Pratylenchus* sp.). Right: following corn; samples averaged 290 nematodes.—Fig. 2. Black shank nursery showing disease development. Left: check variety with resistance similar to Dixie Bright 101. Right: F_5 3044-5.

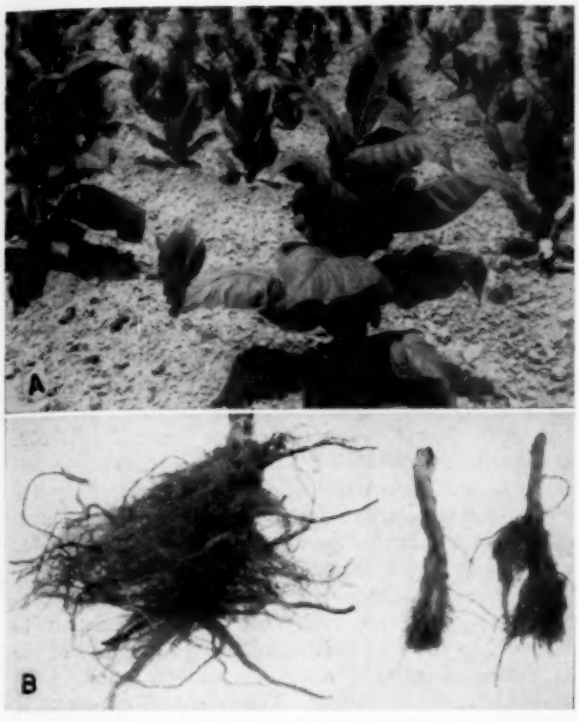


FIG. 3. Growth differences of vigorous Hicks plants and stunted Dixie Bright 101 plants in soil infested with meadow nematode (*Pratylenchus* sp.). A) Tops. B) Roots.

plants developed normally, whereas Dixie Bright 101 plants were stunted in patches varying in size from small areas to whole fields (Fig. 3, A). Meadow nematodes were found in the roots of stunted Dixie Bright 101 plants and stunt nematodes in the soil around the roots[3]. Similar situations have since been observed where accidental mixtures of Hicks and certain other varieties, such as Bottom Special, occur with Dixie Bright 101. In one such field, an average of 1860 meadow nematodes were found in the roots of each Hicks plant with no apparent reduction in top or root growth (Fig. 3); whereas, 80 meadow nematodes per plant caused severe stunting of Dixie Bright 101.

Hicks and Bottom Special are similar varieties of unknown parentage developed by farmer selection. Both have shown vigor under conditions where Dixie Bright 101 was stunted. Although the origin of these varieties is obscure, they are thought to be selections from the variety Orinoco, which has a cultural history dating back to colonial days. They produce good yields of high quality cigarette leaf and have been widely grown on black-shank-free soil. The fact that these varieties came into prominence over numerous others indicates superior adaptibility. Although they are susceptible to black shank, both have been observed to survive longer on black shank test plots than certain susceptible varieties such as 400 (10).

By the fall of 1951 it became apparent that varieties with higher black shank resistance were urgently needed. The most promising approach apparently was to search for such resistance among the several hundred lines and selections already under development. Furthermore, the most suitable place to do the testing and selecting was in fields where moderately resistant Dixie Bright 101 had not survived satisfactorily. Accordingly, such fields were located, and plans made to test all available breeding lines. From 1951 to 1954, 2 families derived from crosses involving Hicks and Bottom Special gave encouraging results. The purpose of this paper is to present an evaluation of the breeding behavior of segregates from these families. A preliminary report already has been given (8).

MATERIALS AND METHODS.—Results presented are limited to those obtained from 2 families, namely, the Hicks (Dixie Bright 102 × Hicks) and Bottom Special (Dixie Bright 101 × (Dixie Bright 102 × Bottom Special)) families. Line 2041 was an F_4 selection from the Hicks family, and line 3006 originated from it. Line 3044 originated from the Bottom Special family as an F_4. F_6 and F_5 selections from the Hicks and Bottom Special families, respectively, were evaluated for yield and for disease resistance during 1954. A family is defined as the lines and selections from a cross or a series of crosses. A selection is the progeny of a single plant. A line is the composite progeny of superior plants from a selection and is propagated by mass selection.

Black shank resistance.—Two fields, in which 75–95 per cent of the Dixie Bright 101 plants had been killed, were obtained in eastern North Carolina for breeding purposes. Plantings consisting of 2 or 3 replicates of 25 plants each were made at each location. Resistant and susceptible checks were planted at intervals of 12 or 15 rows. Counts of diseased plants were taken periodically with the final count being made August 20 to 25. Data are expressed in percentage of plants diseased at final count.

Nematode studies.—Root samples were taken from plants, and soil samples were collected from the rhizosphere. One-pint subsamples of soil were thoroughly mixed and then assayed for nematodes by the Christie and Perry (2) technique. Two-gram samples of roots were placed in a Waring Blendor with 100–125 ml of water and comminuted for 10–15 seconds. This pulp was poured through U. S. 60 and U. S. 200 screens. Nematodes remaining on the 200-mesh screen were washed off and collected in Petri plates for counting.

In 1953, an experiment on the tolerance of breeding lines was set up at McCullers, North Carolina, on soil infested with the root-knot nematode. Duplicate plantings of 3 replicates were made in parallel blocks.

Soil fumigation is known to reduce populations of parasitic nematodes (4) and to increase yields of tobacco. Therefore, in order to provide a check rela-

[3] Samples assayed by T. W. Graham, Pee Dee Experiment Station, Florence, South Carolina.

tively free of nematodes, one block was fumigated by the broadcast method at the rate of 15 gal. per acre of Dowfume W-40 (1,2-dibromoethane). The other was not treated.

Yields.—Yield experiments were conducted on black-shank-free soil at 4 locations in 1953 and at 3 in 1954. Each experiment consisted of 3–4 replicates in a randomized block. Harvested tobacco was cured, sorted, weighed, and assigned official Government grades[4]. Acre values were calculated from these grades and from average auction prices.

EXPERIMENTAL RESULTS.—*Black shank resistance.*—During each of the years 1952 to 1954, inclusive, more than 100 lines and selections were tested at the 2 eastern North Carolina black shank nurseries. The Hicks and Bottom Special families had high black shank resistance. Results for 1952 were similar to those for 1953. Disease data on these families for 1953 and 1954 are presented in Figure 4.

During 1953, Dixie Bright 101 averaged 65 per cent diseased plants, whereas highly resistant Dixie Bright 102 averaged 15 per cent. Susceptible checks, Bottom Special and Hicks, were killed by July 15 before black shank reached its maximum development. Therefore, results are not shown. Lines 2041 and 3006 from the Hicks family were fully as resistant as Dixie Bright 102, whereas 3044 from the Bottom Special family was intermediate in resistance between Dixie Bright 101 and Dixie Bright 102.

Black shank development was more critical in the breeding nurseries during 1954 than in 1953. Over 4 times as many plants of Dixie Bright 102 were diseased as in the previous year, whereas nearly all Dixie Bright 101 plants died. Surviving plants of both varieties were stunted. Root samples from Dixie Bright 102 plants contained root-knot and meadow nematodes (Table 1), and a few stunt nematodes were found in the soil around the roots. Surviving Dixie Bright 101 plants were too badly damaged by black shank to permit an assay of the roots. Root-knot galls were abundant on breeding lines. Expression of black shank symptoms probably was enhanced by unusually dry weather, which placed a moisture stress on plants with roots severely damaged by black shank and nematodes.

The effectiveness of critical selection pressure is shown in Figure 4. Lines 2041, 3006, and 3044 had higher black shank resistance in 1954 than in 1953 as compared to check variety Dixie Bright 102. This is explained on the basis that the 1954 planting comprised the superior plants that survived in 1953. No single-plant selection was any less resistant than its parental line, and most of them were more resistant. Segregation in the Hicks family of F_6 selections was apparent in plant type and is shown by the 1954 disease data. Black shank incidence in 3006-5 was only 1/5 that of its resistant parent, Dixie Bright 102.

TABLE 1.—*Plant parasitic nematodes obtained from 1 pint of soil or 2-g samples of tobacco roots in black shank breeding nursery*

Selection	Number of nematodes found *Pratylenchus* spp.	*Meloidogyne* spp.[a]
3044-5	2820	220
3006-5	80	800
D.B. 102 (Check)	180	380

[a] Larvae only.

Nematode tolerance.—Yield data and root-knot indices of certain lines from both the treated and non-treated root-knot series at McCullers are shown in Figure 6. The root-knot index for Dixie Bright 102, the check, and those for 3 lines were reduced by soil fumigation from a uniformly high to a uniformly low level. The similarity of indices on treated and non-treated soil indicates the nematodes invaded the roots of all lines with equal facility, regardless of the population. This supports the opinion of Gaines and Todd (5) that no variety in use is resistant to invasion by the root-knot nematodes. Responses of the experimental entries to the nematodes following invasion differed markedly. Yields of all except 2041 were reduced (Fig. 6). Whereas the yields of Dixie Bright 102 were reduced 198 lb. per acre, the only effect of nematodes present on 2041 in this test was a severe galling of roots.

Additional evidence of nematode tolerance was obtained on the black-shank plots in 1954. Selections 3006-5 and 3044-5 grew normally and vigorously under conditions where adjacent plots contained stunted and dead plants (Fig. 2). Roots of these normal-appearing plants contained both root-knot and meadow nematodes (Table 1).

Yield.—At 4 locations in 1953, only 3044 averaged significantly higher in yield and value than Dixie Bright 101. Lines 2041 and 3006 yielded slightly more than Dixie Bright 101, whereas Dixie Bright 102 was significantly lower in performance.

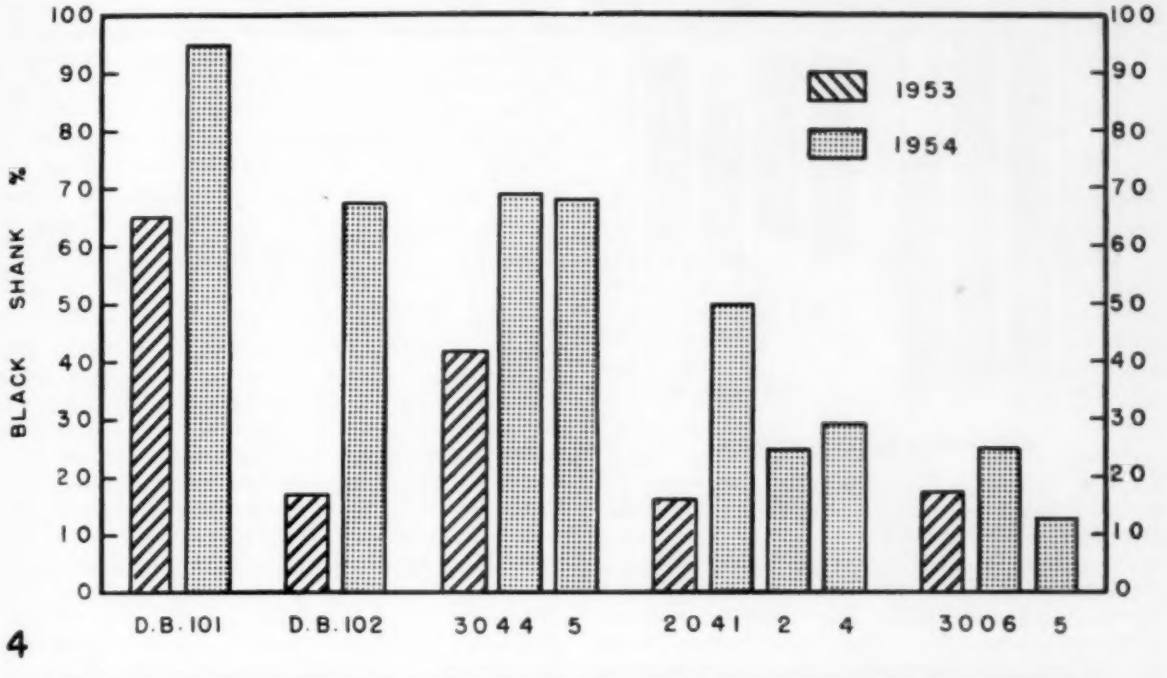
During 1954, at 3 locations, yields and values of all lines and selections averaged higher than those of Dixie Bright 101 (Fig. 5). Yield increases ranged from 13 to 30 per cent, whereas value increases were from 23 to 45 per cent above the check. With the exception of 3044, the percentage-wise increases were higher for acre values than for acre yields. Such increases indicate the new lines produce more of the top quality grades than does Dixie Bright 101[5].

DISCUSSION.—Tobacco growers in North Carolina have for many years planted varieties such as Hicks and Bottom Special, which have resulted from long years of adaptation to the area through continuous selection. Rapid spread of the black shank disease

[4] Courtesy of Tobacco Division, Agricultural Marketing Service, U. S. Department of Agriculture, and North Carolina Department of Agriculture and Division of Markets, cooperating.

[5] Unpublished results from smoke panel tests show the Hicks family of lines have a flavor which would be objectionable in cigarettes.

has caused thousands of growers to change to new black-shank resistant varieties. In general, these new varieties have not proved completely satisfactory with respect to yield and quality. This situation was greatly improved by the release of Dixie Bright 101, which is resistant to black shank, to bacterial wilt, and to Fusarium wilt. In most tests, Dixie Bright 101 gave returns per acre fully equal to those obtained with



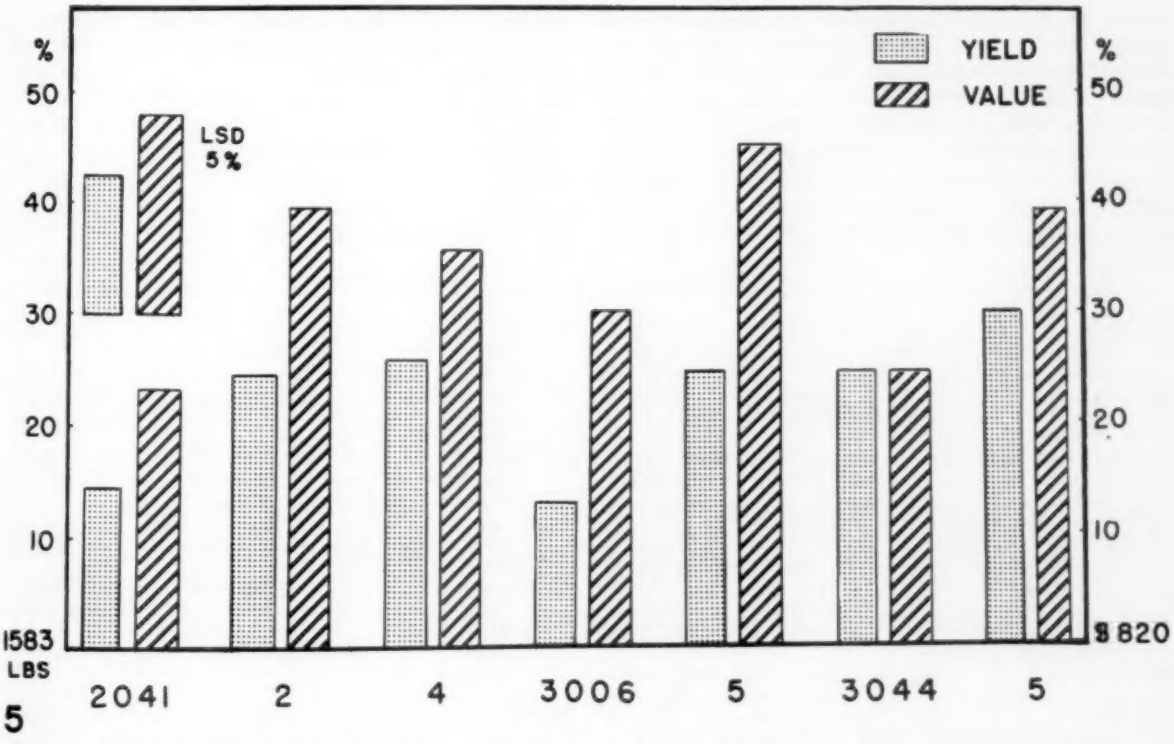


FIG. 4–5. Fig. 4. Incidence of black shank on Bottom Special (3044) and Hicks (2041 and 3006) families of lines and selections and resistant parents, Dixie Bright 101 and Dixie Bright 102.—Fig. 5. Average yields and values from 3 tests during 1954, expressed in percentage increase over Dixie Bright 101.

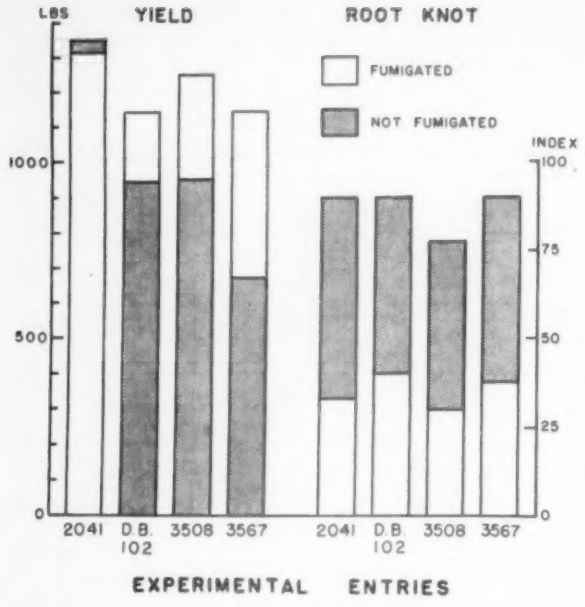


FIG. 6. The influence of soil fumigation on yield and root-knot index. Zero indicates clean roots, and 100 indicates severe galling of all roots.

the better old-line varieties in the absence of black shank. As planting became more extensive, however, it became apparent that at some locations Dixie Bright 101 did not grow vigorously, and it failed to resist attack by the black shank fungus satisfactorily in some fields. It was further observed that in some black-shank-free fields where Dixie Bright 101 grew poorly, varieties such as Hicks and Bottom Special grew vigorously. The studies herein reported have shown that the major cause for weak growth of Dixie Bright 101 was nematode attack, and that rather low root populations of nematodes, particularly meadow nematodes, would greatly retard the growth of this variety. In contrast, the Hicks and Bottom Special varieties tolerated high root populations of these nematodes without appreciable reduction in growth.

At the same time that these host-parasite relations were being investigated, other work was set up to find a practical solution for the failure of Dixie Bright 101 to withstand black shank attack in some fields. To improve resistance, Dixie Bright 102, a low-yielding selection closely related to Dixie Bright 101 but more resistant, was crossed with a wide range of genotypes. The segregating populations were planted under critical disease conditions for selection. Of all the combinations tested, crosses with Hicks and Bottom Special were most promising. Three of these selections, 2041 and 3006-5 out of the Hicks cross and 3044-5 out of the Bottom Special cross, were found to possess a high degree of tolerance to nematodes (Fig. 6, Table 1). These lines, as well as a number of others, combined superior black shank resistance with good yield and quality. Thus, 3006-5, selected out of 2041, showed only a 12 per cent black shank loss under conditions where 68 per cent of the plants were killed in Dixie Bright 102, the resistant parent (Fig. 4). In the 1954 tests, these new lines surpassed Dixie Bright 101 from 13 to 30 per cent in yield and 23 to 44 per cent in estimated value per acre.

The need for conserving valuable disease tolerance from old adapted varieties and for combining this with resistance to new diseases, such as black shank, is generally recognized, but how to accomplish this has not been clear. In this work, these objectives have been attained by crossing with the old varieties and then selecting at field locations where disease development was most critical.

PLANT PATHOLOGY
NORTH CAROLINA STATE COLLEGE
RALEIGH, NORTH CAROLINA
AND
TOBACCO RESEARCH STATION
OXFORD, NORTH CAROLINA

LITERATURE CITED

1. BULLOCK, J. F., AND E. G. MOSS. 1943. Strains of flue-cured tobacco resistant to black shank. U. S. Dept. Agr. Circ. 682, 9p.
2. CHRISTIE, J. R., AND V. G. PERRY. 1951. Removing nematodes from soil. Helminth. Soc. Wash. Proc. **18**: 106-108.
3. CLAYTON, E. E., J. G. GAINES, T. E. SMITH, K. J. SHAW, AND T. W. GRAHAM. 1944. Control of flue-cured tobacco root diseases by crop rotation. U. S. Dept. Agr. Farmers' Bul. 1952. 12p.
4. GAINES, J. G., AND T. W. GRAHAM. 1953. Soil fumigation to control root ills. In Plant diseases. U. S. Dept. Agr. Yearbook. p. 561-567.
5. GAINES, J. G., AND F. A. TODD. 1953. Crop rotations and tobacco. In Plant diseases. U. S. Dept. Agr. Yearbook. p. 553-561.
6. GRAHAM, T. W. 1954. The tobacco stunt nematode in South Carolina. (Abs.) Phytopathology. **44**: 332.
7. JENKINS, W. A. 1948. Strains of flue-cured tobacco resistant to black shank Phytophthora parasitica var. nicotianae and tolerant to certain root-rot complexes. (Abs.) Phytopathology **38**: 15.
8. MOORE, E. L., P. N. DROLSOM, AND E. E. CLAYTON. 1955. High black shank resistance and tolerance to parasitic nematodes in flue-cured tobacco. (Abs.) Phytopathology **45**: 349.
9. MOORE, E. L., G. B. LUCAS, AND E. E. CLAYTON. 1952. Granville wilt and black shank resistant tobacco varieties. N. C. Agr. Exp. Sta. Bul. 378, 18 p.
10. MOSS, E. G., AND J. F. BULLOCK. 1942. Two new varieties of flue-cured tobacco, 400 and 401. N. C. Agr. Exp. Sta. Bul. 337, 8 p.
11. NUSBAUM, C. J. 1951. The distribution of tobacco black shank in North Carolina in 1949 and 1950. U. S. Dept. Agr. Pl. Dis. Rptr. **35**: 358-360.
12. SPARTAN. 1954. New Coker seed resists black shank. Tobacco. Weekly Trade Review (New York). **139** (6): 28-29.
13. WOLF, F. A. 1935. Tobacco diseases and decays. Duke Univ. Press. Durham, N. C. 454 p.

SITE OF DISEASE RESISTANCE INDUCED BY PLANT-GROWTH REGULATORS IN TOMATO[1]

David Davis[2] and A. E. Dimond

SUMMARY

Foliage applications of 2,4-dichlorophenoxyacetic acid, α-naphthaleneacetic acid, or 3-indoleacetic acid reduced the severity of Fusarium wilt of tomato when treatment preceded inoculation. The plant-growth regulators induced resistance in the shoots in the presence of roots as well as in the absence of roots.

The site of natural resistance to Fusarium wilt of tomato has been the subject of a number of investigations. May (7) and Heinze and Andrus (3), using reciprocal grafts of susceptible Bonny Best and resistant Pan American tomato, concluded that resistance is localized in the roots. Keyworth and Dimond (6) noted that root injury resulted in an increase in resistance of Bonny Best tops, and Keyworth (5) concluded that resistance to Fusarium and Verticillium wilts of tomato and hops is dependent on the roots. Snyder et al (9) and Scheffer and Walker (8), however, present evidence that factors for resistance to Fusarium wilt are inherent in both the roots and tops.

In chemotherapy investigations, Davis and Dimond (1) found that resistance to Fusarium wilt was induced in susceptible Bonny Best tomatoes by foliage application of plant-growth regulators (PGR) prior to inoculation. The low *in vitro* fungitoxicity of the PGR and the treatment-inoculation sequence required to induce resistance suggested that the change in susceptibility was due to a modification in the host metabolism unfavorable for disease development. Localization of the site of disease resistance was not established. Since the effects of PGR can be demonstrated in tissues beyond the point of treatment, foliage applications do not preclude action on the root system. Accordingly, experiments were designed to define the area of resistance induced by PGR.

MATERIALS AND METHODS.—The Bonny Best variety of *Lycopersicon esculentum* Mill. was grown at 80°C in sand culture supplemented with Hoagland and Snyder inorganic nutrients (4). At approximately the 8-leaf stage, the tops were dipped in a water solution containing either 10 ppm 2,4-dichlorophenoxyacetic acid (2,4-D), 50 ppm α-napthaleneacetic acid (NAA), or 500 ppm 3-indoleacetic acid (IAA). IAA was reapplied 7 days after the initial IAA treatment. To prevent contamination of the roots with the plant-growth regulators, the tops were inverted until dry.

Within an hour after chemical application, roots were removed from half of the plants within each treatment and the tops placed in 125-ml Erlenmeyer flasks containing 100 ml of the inorganic nutrient solution diluted tenfold. Every second day for 8 days, the tops were placed in fresh diluted nutrient and any roots that had developed on the stem were removed. Eight days after the initial treatment, the roots were removed from the remaining half of the treated plants and all plants were inoculated through the cut end of the stem with a bud cell suspension of *Fusarium oxysporum* f. *lycopersici* Snyd. & Hans. The inoculum was prepared as described by Dimond et al (2) and contained 10^8 cells per ml. The cut ends of the stems were placed in the inoculum for 5 minutes. The inoculated plants were subdivided and handled in the following manner. Half the plants derooted 8 days prior to inoculation were rooted in sand, whereas the remaining half were again placed in 125-ml Erlenmeyer flasks containing 100 ml of the inorganic nutrient solution diluted tenfold. Again, every second day the derooted plants were placed in fresh diluted inorganic nutrient, and any roots that developed on the stems were removed at this time until disease readings were made. The plants derooted at the time of inoculation were divided and handled in a similar fashion.

The pattern of derooting can then be summarized as follows: 1) plants derooted 8 days prior to inoculation and kept free of roots after inoculation, 2) plants derooted 8 days prior to inoculation and rooted after inoculation, 3) plants with roots 8 days prior to inoculation but derooted after inoculation, and 4) plants with intact roots throughout the experiment.

All chemicals were applied prior to inoculation. The plants were covered with a nylon shade cloth to reduce transpiration and thereby prevent desiccation of the derooted shoots. Disease readings were made 3–4 weeks after inoculation, and the disease indices were estimated by the method of Dimond et al (2). Randomized blocks containing 5 plants for each pattern of derooting (equivalent to 20 plants per chemical treatment) were used in each experiment. The experiment was repeated within the same month.

RESULTS.—The plant-growth regulators retarded Fusarium wilt of tomato whether the roots were present or absent either before or after inoculation (Table 1). An analysis of variance of the individual plant scores of the 2 experiments showed that treatments due to chemical treatment were statistically significant.

[1] Accepted for publication May 10, 1956.
We wish to thank Miss Barbara Wooding for technical assistance and Mr. James Eldridge for the statistical analysis of the data. The research reported here was done in cooperation with the Crop Protection Institute.

[2] Present address: Merck and Co., Inc., Rahway, New Jersey.

TABLE 1.—*Disease indices of rooted and derooted tomato plants treated with plant-growth regulators and inoculated with Fusarium oxysporum f. lycopersici*

Experiment	Plants with (+) or without (—) roots[a]		Vascular discoloration indices[b] of plants treated with indicated plant-growth regulators					
	Prior to inoculation	After inoculation	Control	IAA	NAA	2,4-D	Difference Control —IAA	Difference Control —NAA
1	+	+	0.60	0.41	0.05	0.13	0.19	0.57
	—	—	0.57	0.41	0.08		0.16	0.49
	+	—	0.80	0.52	0.32	0.36	0.28	0.48
	—	+	0.73	0.64	0.45	0.13	0.09	0.28
	LSD (5 per cent level)						0.13	0.19
2	+	+	0.69	0.18	0.00	0.17	0.51	0.69
	—	—	0.64	0.08	0.07	0.00	0.56	0.57
	+	—	0.87	0.75	0.50	0.25	0.12	0.37
	—	+	0.78	0.34	0.05	0.06	0.44	0.72
	LSD (5 per cent level)						0.31	0.35

[a] A minus sign indicates that roots were removed 8 days prior to inoculation or at the time of inoculation.
[b] Ratio of the number of internodes showing discoloration of vascular bundles to the total number of internodes per plant. Only the 3 large vascular bundles from the point above the cotyledons to the growing point were used in the disease index estimation. Each figure is based on 5 plants.

Since a number of plants were missing in the final tally of the plants treated with 2,4-D, data of this treatment were not included in the analysis. A least significant difference (LSD) value was calculated (based on the residual error term in the analysis of variance) for the difference in score (across the different root conditions) of the control and of the IAA-treated plants and of the control and NAA-treated plants.

In both experiments, NAA treatment significantly reduced disease regardless of the root condition. IAA treatment was more variable than the NAA treatment; however, the 2 extremes of root conditions, i.e. roots present both before and after inoculation and roots absent both before and after inoculation, produced statistically significant and approximately equal reductions in disease ratings.

Although, as previously stated, the PGR retarded Fusarium wilt whether the roots were present or absent either before or after inoculation, the variability across the 2 experiments was too large to demonstrate whether one root condition was more or less effective than any other root condition in influencing the disease-reducing capacity of the plant-growth regulators.

DISCUSSION.—Resistance to disease was induced in the tops of tomato plants with PGR both in the presence and absence of roots. This experiment demonstrates that plant-growth regulators do not induce resistance to Fusarium wilt solely by producing 1) biochemical or physical changes in the root unfavorable for penetration or development of the pathogen in the roots or 2) a resistance factor that is translocated from the roots to the tops or from the tops to the roots. The possibility remains, however, that treatment of the top with PGR produces changes in the roots equally as effective in reducing disease as those produced in the tops.

Although this study does not define the site of natural resistance to Fusarium wilt, it does suggest that biochemical factors susceptible to change are present in the tops of susceptible tomato plants. Thus, it is possible that natural resistance may result from genetic factors that alter the metabolism of tomato tops in a direction favorable for disease resistance not unlike the factors produced by PGR. An investigation of the physiological changes produced by PGR that are responsible for the increase in resistance of susceptible tomato plants might offer information on natural resistance.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. DAVIS, D., AND A. E. DIMOND. 1953. Inducing disease resistance with plant-growth regulators. Phytopathology **43**: 137-140.
2. DIMOND, A. E., D. DAVIS, E. M. STODDARD, AND R. A. CHAPMAN. 1952. Plant chemotherapy as evaluated by the fusarium wilt assay on tomatoes. Conn. Agr. Exp. Sta. Bul. 557, 82 p.
3. HEINZE, P. H., AND C. F. ANDRUS. 1945. Apparent localization of fusarium wilt resistance in the Pan American tomato. Amer. Jour. Bot. **32**: 62-66.
4. HOAGLAND, D. R., AND W. C. SNYDER. 1933. Nutrition of the strawberry plant under controlled conditions. Amer. Soc. Hort. Sci. Proc. **30**: 288-294.
5. KEYWORTH, W. G. 1953. Verticillium wilt of the hop. VI. The relative roles of root and stem in the determination of wilt severity. Ann. Appl. Biol. **40**: 344-361.
6. KEYWORTH, W. G., AND A. E. DIMOND. 1952. Root injury as a factor in the assessment of chemotherapeutants. Phytopathology **42**: 311-315.
7. MAY, C. 1930. The effect of grafting on resistance and susceptibility of tomatoes to fusarium wilt. Phytopathology **20**: 519-521.
8. SCHEFFER, R. P., AND J. C. WALKER. 1954. Distribution and nature of Fusarium resistance in tomato plant. Phytopathology **44**: 91-101.
9. SNYDER, W. C., K. F. BAKER, AND H. N. HANSEN. 1946. Interpretation of resistance to Fusarium wilt in tomato. Science **103**: 707-708.

ORIGIN AND COMPOSITION OF CELL INCLUSIONS ASSOCIATED WITH CERTAIN TOBACCO AND CRUCIFER VIRUSES [1]

Miguel Rubio

SUMMARY

Cell inclusions associated with infections by certain tobacco and crucifer viruses were studied by means of the light and electron microscopes. The inclusions were found to have diverse modes of formation. Some, such as those of turnip yellow mosaic, result from aggregation of plastids. Others, such as those induced by the cabbage black ring virus, are formed by the aggregation of small refringent particles in the cytoplasm in a manner like that described by Sheffield for tomato aucuba mosaic inclusions. Another type, such as that associated with cauliflower mosaic, appears to be formed by gradual enlargement.

The virus content of cell inclusions also was found to vary greatly. A late stage of the cabbage black ring X-body is composed mainly of flexuous strands detectable under low magnifications of the electron microscope. Under higher magnifications of the electron microscope, these strands were found to be composed mainly of long parallel-arranged fibrils, each fibril having a width that appears to be the same as that of virus particles. These fibrils appear to divide or fragment transversely into elongated flexuous virus particles that may be found free around the strands. Other inclusions, such as the X-bodies of cauliflower mosaic and those induced by the common strain of tobacco mosaic virus, were found to contain few, if any, detectable virus particles and appeared to be composed mainly of amorphous material.

Some inclusions, such as the vesicular inclusions associated with cabbage black ring, appeared to have a distinct surface membrane.

It has long been known that some viruses cause abnormal inclusion bodies to be formed in the cells of the host. Since their first description by Iwanowski (4) in 1903, these bodies have attracted much attention, and their origin and nature have been the subject of considerable study and speculation. In this paper the results of further studies on cell inclusions in virus-infected plants are recorded.

MATERIALS AND METHODS.—Epidermal strips and paraffin sections of healthy and infected leaves were used for these studies. In some preparations, the strips were fixed in Carnoy's alcohol-chloroform-acetic fixing solution (8). In others, the preparations were stained without fixation. After fixation, the preparations usually were washed in distilled water, stained, rinsed in water, and finally mounted in water for study with the light microscope. A 0.5 per cent aqueous solution of phloxine for about 10 minutes was the stain and time duration used most frequently. Other stains used were a Feulgen:light green combination, Giemsa, and phloxine:methyl green. The Sakaguchi reaction for arginine (5) also was used.

For the electron microscope studies, fixed or unfixed epidermal strips were stained with phloxine and placed under the low power of the light microscope. Host cells containing red-stained inclusion bodies were opened with a dissecting needle and the cell contents allowed to flow out into the surrounding water. The desired cell inclusion was taken by means of a capillary pipette under the light microscope and transferred to a collodion-covered grid for electron microscopy. Before it was shadowed with palladium, the grid was examined under the light microscope to determine whether the desired cell inclusion or a portion thereof was over an opening in the grid.

The host used for the common strain of tobacco mosaic virus (TMV) was *Nicotiana tabacum* L. Turnip (*Brassica rapa* L.) and cauliflower (*B. oleracae* var. *botrytis* L.) were used as hosts for cabbage black ring and cauliflower mosaic viruses and Chinese cabbage (*B. pekinensis* (Lour.) Rupr.) for turnip yellow mosaic virus.

Tobacco mosaic inclusions.—Under the electron microscope, tobacco mosaic X-bodies that were sufficiently thin to show internal fine structure were found to be composed mainly or entirely of amorphous material (Fig. 1, A). A few rod-shaped virus particles were found in or under the amorphous material, but they were not sufficiently numerous to indicate the presence of virus particles in the inclusion when it was in the host cell. The finding of little or no virus in these X-bodies is in agreement with the histochemical studies of Rawlins, Weierich, and Schlegel (5) and with the electron microscope studies of sectioned X-bodies by Skotland, Hagedorn, and Stahmann (11). In these 2 studies, no virus was detected in X-bodies. As shown in Figure 1, B, at least certain of the X-bodies have fibrous masses of TMV particles adsorbed on the surface of the body.

In contrast, the X-bodies associated with the aucuba strain of TMV apparently contain much virus. Sheffield (10) was able to obtain considerable amounts of infective virus from washed X-bodies that had been

[1] Accepted for publication May 15, 1956.

This research was conducted in the Department of Plant Pathology, University of California, Berkeley, under a Fulbright Grant from the Institute of International Education of New York. The author wishes to express appreciation to Dr. T. E. Rawlins, under whose supervision this work was done, and to Drs. A. H. Gold, W. N. Takahashi, and C. M. Tompkins for helpful suggestions and for plant materials.

removed from host cells infected by the aucuba strain. Rubio (6) made electron microscope studies of aucuba X-bodies that had been removed from the host and found masses of long parallel fibrils. Each fibril had a width that appeared to be essentially the same as that of TMV particles.

The crystal-like cell inclusions associated with the common strain of TMV apparently have a very different composition from the X-bodies associated with the same strain. Steere and Williams (12) and Rubio (7) made electron microscope studies of these crystal-like inclusions after they had been removed from the host and found that these inclusions are composed mainly or entirely of rod-shaped virus particles.

Cabbage black ring inclusions.—Two types of inclusion, the X-body and the vesicular types, have been found associated with cabbage black ring virus. The X-bodies were described by Rubio (6) and by Berkeley and Weintraub (3). Soon after infection in the present experiments, small granules appeared in the cytoplasm; these granules apparently coalesced to build up X-bodies, as occurs in the X-bodies associated with tomato aucuba mosaic (9). Usually, they did not become vacuolate, but they were granular and later had a fibrous appearance when viewed with a light microscope. Generally they were adjacent to and larger

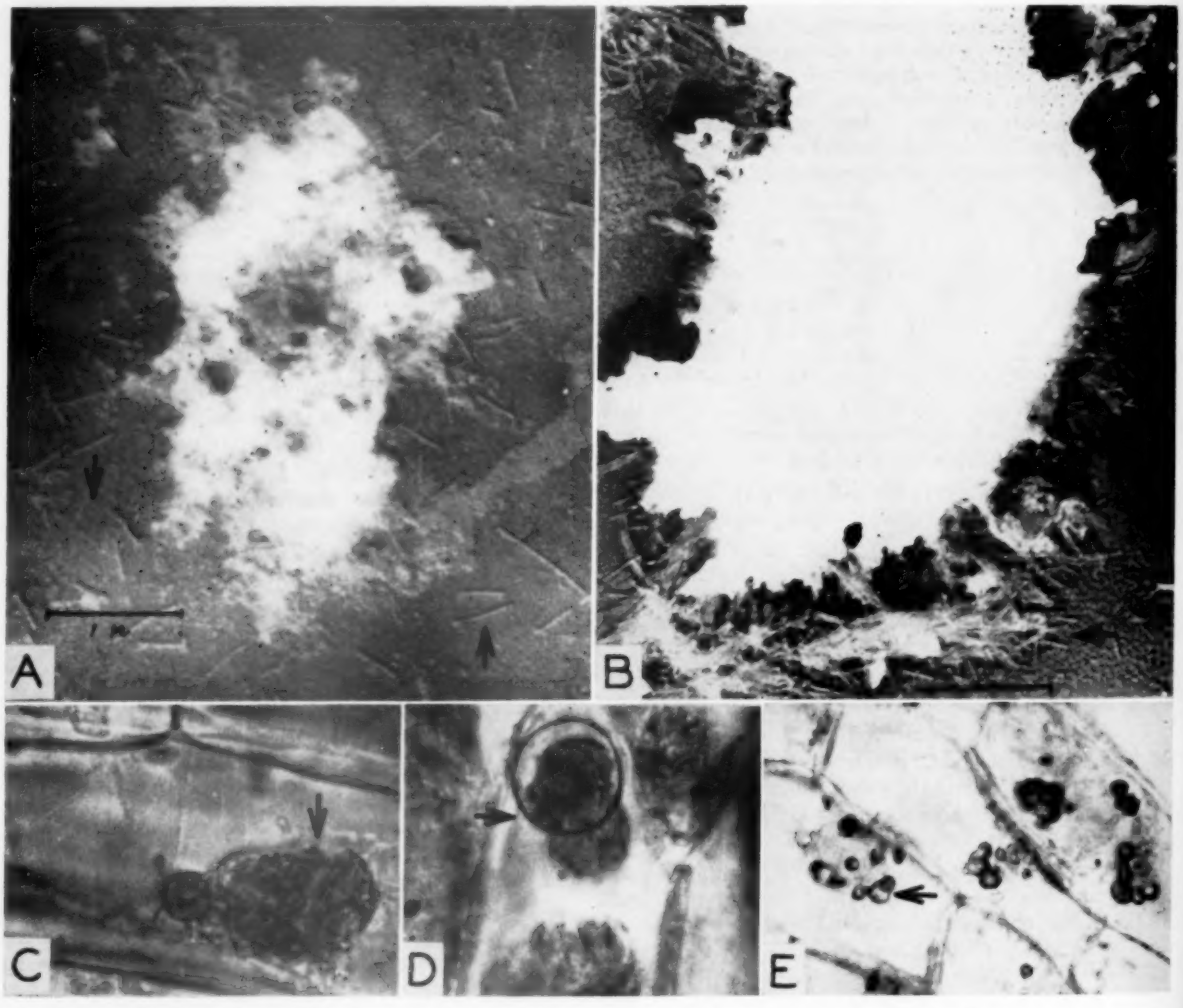


FIG. 1, A) Electron micrograph of a portion of a tobacco mosaic X-body (from a tobacco plant infected by the common strain of TMV) that was sufficiently thin to show its internal structure. The body was composed mainly or entirely of amorphous material and, unlike the strand from a cabbage black ring inclusion shown in Figure 2, A, contained few, if any, virus particles. Rod-shaped virus particles (arrows) were present in the field around the amorphous material. B) Electron micrograph of a tobacco mosaic X-body that was too thick to show internal structure but did have fibrous masses of virus particles (arrow) absorbed to its surface. C) Photomicrograph of a large granular X-body (arrow) of cabbage black ring adjacent to a smaller host nucleus. D) Photomicrograph of a vesicular inclusion of cabbage black ring, showing the distinct membrane (arrow) around the inclusion. E) Photomicrograph of an epidermal strip containing numerous darkly stained, refringent inclusions of cauliflower mosaic. In 1 cell, the inclusions (arrow) were clearly grouped around a nucleus.

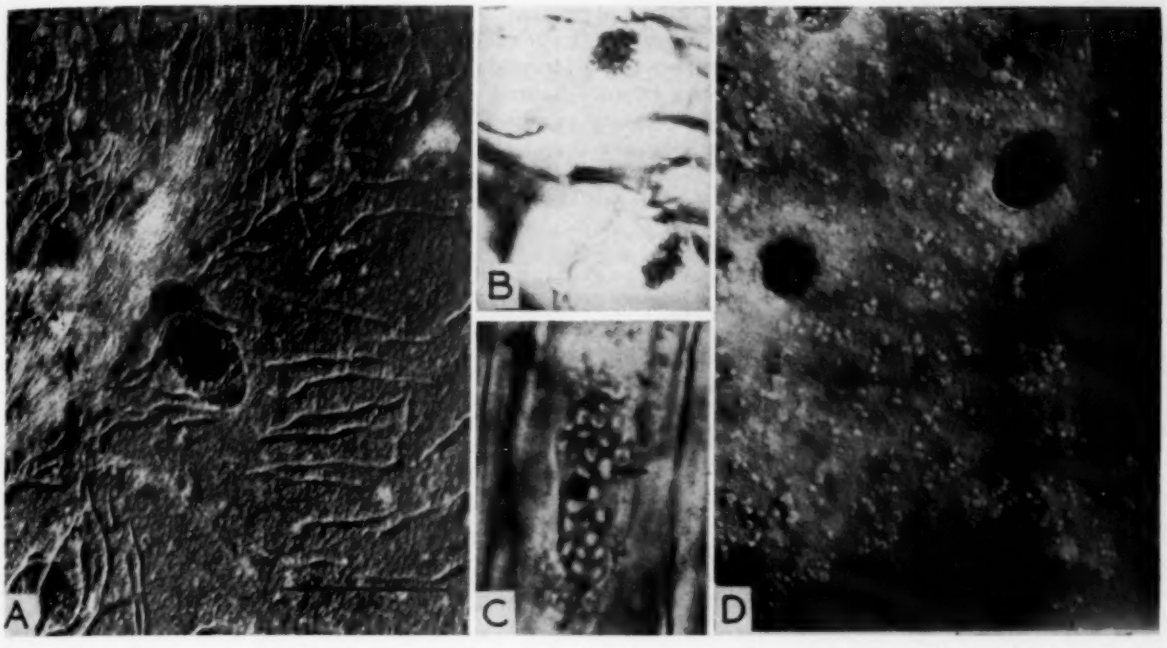


FIG. 2, A) Electron micrograph of a portion of a strand (arrow) from a cabbage black ring X-body, showing that the strand was composed mainly of parallel fibrils, each of which was approximately the width of virus particles. The lower right portion of the field shows numerous isolated flexuous rods that are thought to be virus particles that had become detached from the strand. B) Photomicrograph of early stages of 2 turnip yellow mosaic X-bodies in which a number of plastids had aggregated to form the bodies. C) Photomicrograph of a later stage of a turnip yellow mosaic X-body that contained numerous vacuoles (arrow). D) Electron micrograph of a late stage of a portion of a turnip yellow mosaic X-body, showing numerous more-or-less spherical particles of various sizes. All structures characteristic of plastids have disappeared. ×23000.

than the nucleus and were ovoid in form (Fig. 1, C). These X-bodies gave the Millon reaction for proteins and the Sakaguchi (5) reaction for arginine. They did not give the Feulgen reaction for desoxyribonucleic acid.

Under the lower magnifications of the electron microscope, the late stages of these X-bodies showed the presence of flexuous strands. Under higher magnifications, these strands were found to be made up mainly of parallel long fibrils (Fig. 2, A); each had a width that appeared to be essentially the same as that of isolated virus particles. These fibrils appeared to divide or fragment transversely into flexuous virus particles, which could be found free around the strands (Fig. 2,A). There appeared to be some amorphous material present between the fibrils.

The vesicular cell inclusions were found in a late stage of infection when the host plants showed necrotic lesions. They were spherical or ovoid in form and had a distinct outer membrane (Fig. 1,D). In the early stages, the contents of these inclusions appeared to be a clear liquid containing scattered refringent particles. Later, an angular crystal-like body apparently was formed, and it filled most of the space within the membrane. These angular structures were a strong yellow color. In a later stage, the membrane disappeared, leaving the crystal-like body intact. The yellow color spread through the cells, and the host cells finally became necrotic. The vesicular bodies did not give a positive reaction with the Feulgen test for desoxyribonucleic acid. These bodies were not studied under the electron microscope.

Cauliflower mosaic inclusions.—The inclusion bodies associated with cauliflower mosaic virus have been described briefly by Rubio (6). In the present studies, they were found to be of the noncrystalline, X-body type and were usually spherical or ovoid but sometimes were of irregular shape. Their outline was sharp, and they were very refringent but never vacuolate. They seemed to have a smooth external membrane. One to 8 inclusion bodies usually were found in a single cell, but occasionally more were found. In most cases they were adjacent to the nucleus. They appeared in the cells of the host plants 3–4 weeks after infection, when the external symptoms were well developed. Those shown in Figure 1,E are considerably smaller than the nucleus, but some inclusions may become somewhat larger than the nucleus. They seemed to develop by a gradual increase in size. No coalescing of 2 or more bodies to form a single body was detected. These inclusions were not soluble in water, alcohol, chloroform, or xylol. They withstood low pH (pH 2-3) but swelled if treated with 10 per cent NaOH. They gave a positive Millon reaction for proteins and stained well with phloxine but not with the Fuelgen stain; they could be removed

intact from the cell without previous fixation and could be handled with a needle. Electron microscope studies failed to reveal the presence of virus particles associated with these cell inclusions. Other electron microscope studies by Rubio (6) and Bawden and Nixon (1) also have failed to reveal the virus particles associated with this virus.

Turnip yellow mosaic inclusions.—The inclusions associated with this disease were of the noncrystalline X-body type, often very compact and granular, but sometimes vacuolate or reticulate. Their shape varied but was generally ovoid. They were larger than the nuclei and were often adjacent to them. Only 1 inclusion body generally was found in each cell. The mature inclusions were found in advanced stages of the disease when the host plant showed very severe chlorosis; earlier, only intermediate forms could be found. The inclusions apparently were formed from coalesced plastids or chloroplasts. In epidermal strips of infected plants, without stain or after staining with phloxine without fixation, the different stages of inclusion body formation could be seen under the light microscope. In normal cells, the plastids were evenly distributed in the cytoplasm. In cells affected by the virus, a clump of plastids could be seen (Fig. 2,B). In the early stages, the structure of the plastids appeared normal and they did not stain with phloxine. Later, the plastids in the clump began to disintegrate and the clump became more compact and granular and stained pink with phloxine. The last stage was characterized by the complete degeneration of the plastids to form a granular or vacuolate body (Fig. 2,C), that stained strongly with phloxine. The inclusions gave the Millon reaction for proteins. After treatment of the epidermal strips with a 10 per cent solution of NaOH, the normal plastids were not visibly affected, whereas the clumps of plastids that had degenerated sufficiently to stain with phloxine were decomposed in a few minutes by the alkali.

Under the electron microscope, the inclusions again were found to be made of plastids in different stages of degeneration and to contain spherical particles of different sizes (Fig. 2,D).

Instituto de Edafologia y Fisiologia Vegetal
Madrid, Spain

LITERATURE CITED

1. Bawden, F. C., and N. L. Nixon. 1951. The application of electron microscopy to the study of plant viruses in unpurified plant extracts. Jour. Gen. Microbiol. **5**: 104-109.
2. Bawden, F. C., and F. M. L. Sheffield. 1939. The intracellular inclusions of some plant virus diseases. Ann. Appl. Biol. **26**: 102-115.
3. Berkeley, G. H., and M. Weintraub. 1952. Turnip mosaic. Phytopathology **42**: 258-260.
4. Iwanowski, D. 1903. Über die Mosaikkrankheit der Tabakspflanze. Ztschr. f. Pflanzenkrank. **13**: 1-41.
5. Rawlins, T. E., A. J. Weierich, and D. E. Schlegel. 1956. A histochemical study of certain plant viruses by means of the Sakaguchi reaction for arginine. Virology. **21**: 308-311.
6. Rubio-Huertos, M. 1950. Estudios sobre inclusiones intracelulares producidas por virus en las plantas. Microbiol. Española **3**: 207-232.
7. Rubio-Huertos, M. 1954. Rapid extraction of intact crystalline inclusions from the cells of plants infected with tobacco mosaic virus. Nature [London] **174**: 313.
8. Sass, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill, New York.
9. Sheffield, F. M. L. 1931. The formation of intracellular inclusions in solanaceous hosts infected with aucuba mosaic of tomato. Ann. Appl. Biol. **4**: 471-493.
10. Sheffield, F. M. L. 1939. Micrurgical studies on virus-infected plants. Roy. Soc. London, Proc., Ser. B. **126**: 529-538.
11. Skotland, C. B., D. J. Hagedorn, and M. A. Stahmann. 1955. Electron microscopy of tobacco mosaic virus in situ. Phytopathology **45**: 603-607.
12. Steere, R. L., and R. C. Williams. 1953. Identification of crystalline inclusion bodies extracted intact from plant cells infected with tobacco mosaic virus. Amer. Jour. Bot. **40**: 81-84.

SUSCEPTS, INDICATORS, AND FILTERS OF TRISTEZA VIRUS, AND SOME DIFFERENCES BETWEEN TRISTEZA IN ARGENTINA AND IN FLORIDA [1]

L. C. Knorr [2]

SUMMARY

Tristeza in Argentina is compared with the disease of the same name in Florida. The production of vein-clearing symptoms in seedlings of Key lime (*Citrus aurantifolia* (Christm.) Swing.), the destruction of sweet orange trees on sour orange rootstocks, the occurrence of stem pitting and honeycombing in susceptible tissues, and the presence of a characteristic involvement of vascular tissues at the bud union have suggested that the 2 diseases are identical. On the contrary, differences in host ranges, growth rates of infected plants, and specificity of the stem-pitting reaction suggest that the causal viruses or virus complexes in the 2 areas are not the same. Argentine tristeza virus produces vein clearing in *Aeglopsis chevalieri* Swing., severe stunting in Key lime, and ropy trunk and wood pitting in the trunks of grapefruit; Florida tristeza virus, in contrast, produces no reaction in *A. chevalieri*, no severe stunting in Key lime, and no ropy trunk or wood pitting in trunks of grapefruit.

Host-range studies of Argentine tristeza virus disclosed the existence of 4 indicator plants not previously reported, viz., *Afraegle paniculata* (Schum.) Engl., *Pamburus missiones* (Wt.) Swing., *Citrus combava* Raf., and *C. hystrix* DC. Of these, the last mentioned proved also to be an indicator of Florida tristeza virus.

A. chevalieri was found to be a filter plant useful in separating psorosis virus from admixtures with Florida tristeza virus.

Studies carried out in Argentina from 1950 to 1952 and subsequently in Florida have extended the known suscept range of tristeza virus, revealed the presence of significant differences between tristeza in Argentina and in Florida, and demonstrated a filter plant for separating psorosis virus from Florida tristeza virus. Details concerning these findings, the procedures followed in obtaining them, and the attendant consequences, are presented herein.

At the Tristeza Laboratory of the Florida State Plant Board in Concordia, E. R., Argentina, 39 species of plants were tested for susceptibility to tristeza in attempts to discover, primarily, what species might introduce the causal virus into areas protected by quarantines and, secondarily, what species might serve as indicator, differential, or filter hosts. Rutaceous as well as nonrutaceous plants were tested. Seedlings were reared under insect-free conditions. From 2 to 21 seedlings, usually 4, of each species were infested with 10–50, usually 25, individuals of the highly efficient vector, *Aphis citricidus* Kirk., collected from naturally infected grove citrus trees. Periodically these aphids were tested and shown to be viruliferous by letting them feed on Key lime indicator plants. Of the check plants, usually 5 per species, some were infested with nonviruliferous aphids, and some were not infested.

Following insect feeding, plants were examined periodically for differences between those treated and those untreated. Species of the genus *Citrus* were observed at weekly intervals for specific symptoms that might have made possible a diagnosis of commercial varieties in the field. Species of other genera were inspected at monthly intervals. Observation of most series was continued for 3–10 months. Results are reported only for those species on which aphids lived for a period (usually 2 days) believed to be sufficient to effect transmission.

Of the 39 species, 8 (all rutaceous) were found to exhibit specific symptoms of tristeza (Table 1). Four have not previously been reported to be suscepts: *Afraegle paniculata* (Schum.) Engl., *Pamburus missiones* (Wt.) Swing., *Citrus hystrix* DC., and *Citrus combava* Raf. Symptoms in these plants resemble symptoms produced by Argentine tristeza in Key lime

TABLE 1.—*Indicator plants of the tristeza virus as determined in Argentina in tests in which the vector Aphis citricidus Kirk. was used as inoculating agent*

Species	Symptoms produced[a]
Aeglopsis chevalieri Swing.	VC, ST, SP
Afraegle paniculata (Schum.) Engl.	VC
Citrus aurantifolia (Christm.) Swing.	VC, ST, SP
Citrus combava Raf.	VC
Citrus hystrix DC.	VC, SP
Citrus paradisi Macf.	SP
Citrus sinensis (L.) Osbeck	SP[b]
Pamburus missiones (Wt.) Swing.	VC

[a] VC = vein clearing, similar to the reaction caused by tristeza virus in the immature leaves of Key lime indicator plants. ST = stunting of over-all growth of plant. SP = stem pitting in the wood of decorticated stems.

[b] In occasional plants only.

[1] Accepted for publication May 16, 1956.
Florida Agricultural Experiment Stations Journal Series, No. 482.

[2] Associate Plant Pathologist, Citrus Experiment Station, University of Florida, Lake Alfred, Florida. The writer acknowledges the considerable assistance rendered by many in the collecting and dispatching of seeds used in these trials. This note is in appreciation to F. B. Brown, Kuala Lumpur, Malaya; H. Chapot, Rabat, French Morocco; I. D. Clement, Cienfuegos, Cuba; B. Gueroult, Abidjan, Ivory Coast; D. G. Huttleston, Brooklyn, New York; J. E. Mignard, Kindia, French Guinea; F. D. Venning, Havana, Cuba; and the late David Fairchild, "The Kampong," Florida.

indicator plants: 1) chlorotic spots and dashes along the lateral veins and veinlets, best seen in immature leaves when viewed with transmitted light; 2) pits and grooves in the decorticated woody cylinder; and 3) stunting of the plant as a whole.

Of the remaining 31 species, none showed symptoms considered to be specific for, nor characteristic of, tristeza. This group of nonreactors included 17 rutaceous species (*Aegle marmelos* (L.) Corr., *Atalantia ceylanica* (Arn.) Oliv., *A. citroides* Pierre ex Guill., *Casimiroa edulis* La Llave, *Citrus grandis* (L.) Osbeck, *C. limon* (L.) Burm. f., *C. reticulata* Blanco, *Evodia* sp., *Glycosmis citrifolia* (Willd.) Lindley, *Murraya koenigii* (L.) Spreng., *Ptelea trifoliata* L., *Ruta chalpensis* L., *R. graveolens* L., *Severinia buxifolia* (Poir.) Tenore, *S. disticha* (Blanco) Swing., *Swinglea glutinosa* (Blanco) Merr., and *Triphasia trifoliata* (Burm. f.) P. Wilson) and 14 nonrutaceous species (*Beta vulgaris* L., *Brassica oleracea* L., *Calendula* sp., *Capsicum* sp., *Celosia* sp., *Cucumis sativus* L., *Datura* sp., *Dianthus caryophyllus* L., *Iberis* sp., *Lactuca sativa* L., *Luffa* sp., *Nicotiana tabacum* L., *Ocimum basilicum* L., and *Zinnia* sp.).

Because of the unexpected closing of the Argentine Tristeza Laboratory, only a few of the inoculated but nonreacting plants were tested for the possibility that they might act as masked carriers of tristeza virus. In index tests on Key lime seedlings, inocula from plants of *Murraya koenigii*, *Glycosmis citrifolia*, *Atalantia ceylanica*, and *Severinia disticha* failed to induce symptoms, indicating that these species are insusceptible to tristeza virus.

The similarities between Argentine tristeza and Florida tristeza have led to the generally accepted hypothesis (5) that these diseases are caused either by the same virus or by 2 very closely related viruses. Host-range studies reported here emphasize certain differences between Argentine and Florida tristeza viruses. One of these differences involves the susceptibility of *Aeglopsis chevalieri*. When infected with Argentine strains, this plant responds quickly and sharply with vein clearing in the foliage, stem pitting in the wood, and stunting of the plant as a whole (Fig. 1, A). Reactions in *A. chevalieri* are so conspicuous, in fact, that this species is considered a better indicator plant under Argentine conditions than is Key lime (*Citrus aurantifolia* (Christm.) Swing.). In Florida, on the contrary, *A. chevalieri* has proved to be insusceptible. Seeds of this species (derived from the same parent tree as that which supplied seeds for the Argentine trials) were planted and grown under insect-free conditions. Forty of the resultant seedlings were selected for uniformity and divided into 4 groups of 10 plants. Each group was budded with a different source of tristeza virus. Actual presence of virus in the various sources of budwood was confirmed by index tests on Key lime in which the vein-clearing symptoms typical of tristeza were obtained. Despite positive reactions in Key lime, none of the 40 aeglopsis seedlings grafted with the same sources have, in the course of 2½ years, developed symptoms—neither vein clearing, stem pitting, nor stunting.

To check the possibility that symptoms in *A. chevalieri* in Florida may be masked because of environmental influences, 9 of the 40 aeglopsis seedlings originally budded with 4 different sources of Florida tristeza virus were indexed into Key lime seedlings with negative results, indicating that the aeglopsis seedlings were not masked carriers.

A second difference between Argentine and Florida

FIG. 1. The stunting effect of Argentine tristeza virus A) in *Aeglopsis chevalieri* and B) in *Citrus aurantifolia* cult. (Key lime seedlings). Plants to the left in each figure exemplify normal growth, having been infested with nonviruliferous aphids, whereas those at the right illustrate the degree of stunting resulting 70 and 50 days, respectively, after infestation with viruliferous aphids.

tristeza virus is reflected in the growth rates of inoculated Key lime seedlings. In Argentina, stunting usually appears within 30 days following inoculation, infected plants being half the size of healthy checks within 50 days (Fig. 1, B). In Florida, in contrast, there is no appreciable difference in the size of infected plants and that of noninfected ones, even after 3 years. This failure to produce severe stunting in Key lime is common to all strains of the virus tested in Florida. To date, 111 tristeza-affected trees from various parts of the state have been indexed in Key lime without subsequent development of any conspicuous stunting of the latter.

Another difference between Argentine and Florida tristeza relates to the development of stem-pitting symptoms. Whereas both types commonly show pitting in the wood of twigs in such varieties as Key and Tahiti lime, Florida and Argentine forms of tristeza react differently in trunks of grapefruit trees. In Argentina (4, 6) as well as in Brazil (3), South Africa (8), and Australia (1), a distinctive stem-pitting syndrome is found in trunks of grapefruit trees affected with tristeza virus. Externally, symptoms consist of longitudinal grooves that extend from the bud union into the main limbs. These channels and the interposing ridges give affected trunks the appearance of being sheathed in a bundle of vertically arranged ropes (Fig. 2, A)—an aspect that suggests the name "ropy trunk" for this symptom. When bark is removed from such areas, the woody cylinder is seen to be heavily pitted. The depressions measure 10–60 mm in length and 1–8 mm in width; when viewed collectively, they are suggestive of the vermiculated tunnels of wood borers (Fig. 2, B). In Florida, contrariwise, ropy trunk has not been encountered, despite the fact that many grapefruit trees are known to contain tristeza virus, and only in a few instances have grapefruit trees been observed in which there is even a pitting in the twigs. In California, grapefruit trees are singularly unaffected by trunk pitting. Of 2042 citrus trees examined by Bitters (2), half of which were bud-inoculated with quick decline virus and the other half of which apparently were inoculated in the field by aphids, stem pitting occurred in only 2.9 per cent of all grapefruit trees inspected (representing 6.6 per cent of the trees examined). It is questionable, though, whether these trees were actually affected by the stem-pitting disease of Oberholzer (8). Varieties reported by Bitters to be heavily pitted (e.g. sour oranges, 13.0 per cent; trifoliate oranges, 76.9 per cent; trifoliate hybrids, 45.9 per cent) failed to show pitting in Argentina under conditions where stem pitting of grapefruit abounded (6).

There is but little knowledge from which to derive a hypothesis that would account for these substantial differences between tristeza in Florida and tristeza in Argentina. Perhaps they are due to variations in environmental conditions or to differences in the virus strains or virus complexes that are involved. Still another possibility is suggested by an apparent association between the presence of *Aphis citricidus*, the efficient vector of tristeza, and the occurrence of Argentine tristeza. *A. citricidus* exists in all areas such as Brazil, Uruguay, South Africa, and Australia, where Argentine tristeza is known to occur, but this same vector has not been found in Florida where only the Florida type of tristeza occurs. Neither has *A. citricidus* been reported from California. There, quick decline resembles much more the Florida tristeza than it does the form in Argentina. It is possible that *A. citricidus* not only serves as an efficient disseminator of tristeza but that it also is involved in the pathogenicity of the virus or in its ability to select more virulent strains from the population.

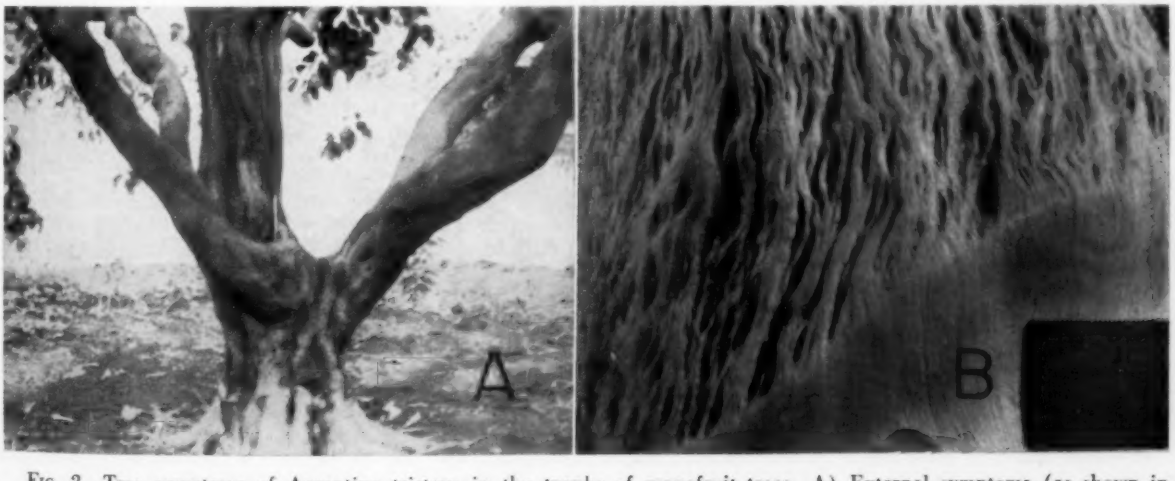


FIG. 2. Two symptoms of Argentine tristeza in the trunks of grapefruit trees. A) External symptoms (as shown in a 23-year-old grapefruit tree on rough lemon rootstock) consist of longitudinal channels in the scion portion of the tree. The appearance of these malformations suggests the name "ropy trunk" for this aspect of the stem-pitting syndrome. B) Removal of the bark from the bud union area of a ropy-trunk affected tree reveals the presence of longitudinal depressions in the woody cylinder. Only the grapefruit portion of the trunk is affected; the sweet orange rootstock portion below the bud union remains symptomless. ×1.6.

Throughout this paper, the name Florida tristeza has been used. Selection of this term was motivated by considerations of convenience and by a reluctance to equate Florida tristeza with similar diseases elsewhere in the absence of cross-inoculation experiments. It is believed, however, on the basis of published symptomatologies, that the disease in Florida is similar to or identical with the one present in California, whereas both differ in much the same respects from tristeza as it occurs in Argentina. If subsequent research should establish the existence of specific differences in the causal agents in Florida and in Argentina, it may become more appropriate to refer to the disease in Florida as "quick decline"—the name used since 1939 for the disease in California. This suggestion would be consonant with one made by McClean and van der Plank (7) that the term tristeza is more properly applied to the disease caused by a virus complex consisting of a seedling-yellows component and a stem-pitting component. Results of indexing trials in Florida have not to date revealed the presence of a seedling-yellows factor in this state.

Recent evidence indicates that tristeza virus has been in Florida for nearly 50 years. That it may have been introduced with the Meyer lemon from China about 1908 is indicated by the fact that tristeza virus was found recently in all of 25 Meyer lemon trees selected at random from different parts of the State. Additional evidence of its long presence in Florida is deduced from observations in a block of Surprise Navel orange trees approximately 65 years old that is surrounded by Valencia orange trees, all on sour orange rootstock, planted about 35 years ago. Individual Navel orange trees in the block were found recently to be infected by tristeza virus; adjacent Navel trees were found to be free of the virus. Moreover, none of the surrounding Valencia orange trees showed evidence of tristeza; neither did Key lime seedlings that were inoculated by means of buds from 6 of these trees. It seems highly unlikely that such a distribution of virus could be the result of insect transmission during recent years; it seems more likely that the infected Navel orange trees harbored the virus when first planted 65 years ago.

Despite its long presence in Florida, tristeza went unrecognized. Losses produced by Florida tristeza were apparently insufficient to suggest a relationship with the disease that produced such catastrophic losses in South America. Thus, it was not until 1952, when Grant (5) observed vein clearing in Key lime seedlings that had been budded with wood from Florida orange trees in a state of decline, that a relationship to Argentine tristeza became apparent. Because of the tendency to define tristeza in terms of damage characteristic of the disease in Argentina, it may be that Florida-type tristeza is being overlooked in areas of the world still regarded as free of this disease, just as it was overlooked for many years in Florida.

In the course of host-range studies in Florida, it was found that Chevalier's aeglopsis (*A. chevalieri*), while not an indicator plant for Florida tristeza virus, did function as a filter plant. Usually when a mixture of tristeza virus and psorosis virus is budded into Key lime seedlings, there follows a strong development of vein clearing (indicative of tristeza virus) and a weak development of flecking (indicative of psorosis virus). In 9 tests, when budwood containing a mixture of these 2 viruses was budded into seedlings of *A. chevalieri* and these plants were later indexed to Key lime and sweet orange, only psorosis developed, indicating that the aeglopsis acts as filter host that removes tristeza virus and allows passage of the psorosis virus alone.

Conduct of the work in Argentina offered an excellent opportunity to examine the host range of tristeza virus, for there a highly efficient vector could be used to inoculate plants other than the Rutaceae. In contrast, host-range studies in Florida have been hampered by the absence of an efficient vector, thereby making it necessary to resort to grafting. No success has yet attended efforts at interfamilial grafting.

CITRUS EXPERIMENT STATION
UNIVERSITY OF FLORIDA
LAKE ALFRED, FLORIDA

LITERATURE CITED

1. ANON. 1950. Stunt bush, or stem pitting: a potentially serious disease of Marsh grapefruit. Agr. Gaz. N. S. Wales **61**: 365-366.
2. BITTERS, W. P., N. W. DUKESHIRE, AND J. A. BRUSCA. 1953. Stem pitting and quick decline symptoms as related to rootstock combinations. Citrus Leaves **33**(2): 8-9, 38.
3. COSTA, A. S., T. J. GRANT, AND S. MOREIRA. 1950. A possible relationship between tristeza and the stem-pitting disease of grapefruit in Africa. Calif. Citrog. **35**: 504, 526-528.
4. DUCHARME, E. P., AND L. C. KNORR. 1954. Vascular pits and pegs associated with diseases in Citrus. U. S. Dept. Agr. Pl. Dis. Rptr. **38**: 127-142.
5. GRANT, T. J., AND H. SCHNEIDER. 1953. Initial evidence of the presence of tristeza, or quick decline, of citrus in Florida. Phytopathology **43**: 51-52.
6. KNORR, L. C., E. P. DUCHARME, AND A. BANFI. 1951. The occurrence and effects of "stem pitting" in Argentine grapefruit groves. Citrus Mag. **14**(2): 32-36.
7. MCCLEAN, A. P. D., AND J. E. VAN DER PLANK. 1955. The role of seedling yellows and stem pitting in tristeza of citrus. Phytopathology **45**: 222-224.
8. OBERHOLZER, P. C. J., I. MATHEWS, AND S. F. STIEMIE. 1949. The decline of grapefruit trees in South Africa. A preliminary report on so-called "stem pitting." So. Africa Dept. Agr. and Forestry. Sci. Bul. 297, 18 p.

UPPER ALTITUDINAL LIMITS OF LODGEPOLE PINE DWARFMISTLETOE IN THE CENTRAL ROCKY MOUNTAINS [1]

Frank G. Hawksworth [2]

SUMMARY

Observations in lodgepole pine stands in Colorado and Wyoming indicate that the upper altitudinal limits of dwarfmistletoe, *Arceuthobium americanum* Nutt., are 200 to 600 ft. below the upper limits of commercial lodgepole pine.

This is the first of a series of articles dealing with the effect of environmental factors on the incidence and abundance of dwarfmistletoes, *Arceuthobium* spp.

A. americanum Nutt. is a widespread and important pathogen of lodgepole pine, *Pinus contorta* Dougl., in the central Rocky Mountains. This note records observations indicating that the upper altitudinal limits of *A. americanum* are a few hundred feet below the upper limits of commercial[3] lodgepole pine.

The European fir mistletoe, *Viscum album* f. sp. *abietis Tubeuf*, has definite upper altitudinal limits (5, 6). Although no such relationship has been reported for the American mistletoes, *Phoradendron* spp., a number of publications indicate that they are very susceptible to extreme cold or to unseasonable drops in temperature. The Eastern mistletoe, *P. flavescens* Nutt., has suffered heavy losses because of low temperature (1, 2, 3, 7), and the juniper mistletoe, *P. juniperinum* Engelm., has been killed by late spring frosts that did not injure the host trees (4). Although little information on this subject is available for the dwarf-mistletoes, it is believed that they are also more susceptible to extreme or unseasonable cold than are their hosts. Drake[4] reports that the dwarfmistletoe, *Arceuthobium campylopodum* f. *tsugensis* Gill, on western hemlock in southeastern Alaska is not found above 500 ft. elevation.

During a dwarfmistletoe-damage survey of the lodgepole pine areas in Colorado and Wyoming, the upper altitudinal limits of *A. americanum* were found to be lower than the upper limits of commercial lodgepole pine (Fig. 1). The highest elevation at which infected stands were found ranged from 9,200 ft. in northern Wyoming to 10,700 ft. in central Colorado.

A dwarfmistletoe-free zone was found consistently at altitudes from 200 to 600 ft. below the upper limits of commercial lodgepole pine. The highest elevations at which infected stands were found (Fig. 1) refer to the extreme upper limits observed; at these elevations only isolated infected groups were found. The highest stands of generalized dwarfmistletoe infection lie 100 to 200 ft. below these upper limits.

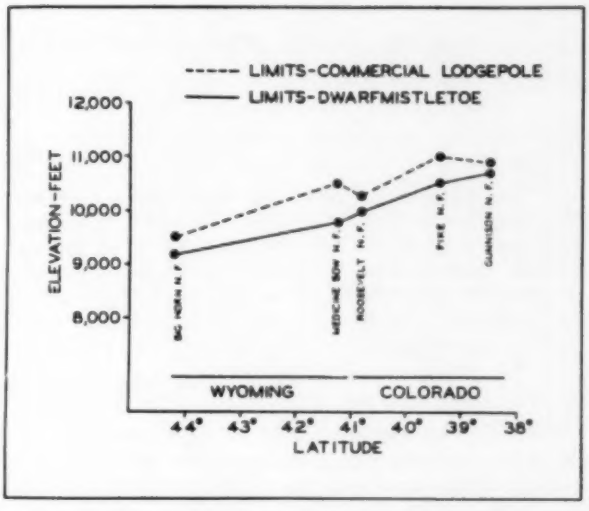


FIG. 1. Upper altitudinal limits of dwarfmistletoe and commercial lodgepole pine stands. The dots indicate the highest points observed in the 5 National Forests.

The elevations given in Figure 1 were the highest found at scattered observation points in the designated forests; no systematic search was made in each locality to find the extreme upper limits of either infected stands or commercial stands.

Low temperatures are presumed to be the primary factor limiting the distribution of dwarfmistletoe in the higher altitudes. The upper limits of infection shown in Figure 1 coincide approximately with the 30°F mean annual temperature isotherm; the minimum temperatures at these points average about −55°F.

Although for the region as a whole the proportion of the lodgepole pine forests in this dwarfmistletoe-free zone is relatively small, on some management units a sizeable proportion of the lodgepole pine stands are involved. On the Centennial Ranger District in the Medicine Bow National Forest, for example, nearly a fourth of the lodgepole pine stands are above 9,800 feet in elevation and hence are non-

[1] Accepted for publication May 23, 1956.

[2] Plant Pathologist, Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, with central headquarters at Fort Collins, Colorado, in cooperation with Colorado A. and M. College.

[3] The term "commercial" as here used refers to stands that support volumes sufficient to warrant logging.

[4] Drake, G. L. 1915. Mistletoe infection in S.E. Alaska and its importance in treatment of hemlock stands. Ann. Silvical Rpt. Tongass National Forest.

infected; the incidence of dwarfmistletoe in stands below this altitude is about 60 per cent on an area basis.

ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION
ALBUQUERQUE, NEW MEXICO

LITERATURE CITED

1. BRAY, W. L. 1910. The mistletoe pest in the Southwest. U.S. Dept. Agr., Bur. Pl. Indus. Bul. 166, 39 p.
2. GARMAN, H. 1913. The woody plants of Kentucky. Ky. Agr. Exp. Sta. Bul. 169, 29 p.
3. GOULD, C. N. 1901. Range of mistletoe in Oklahoma. Plant World **4**: 32.
4. PHILLIPS, F. J. 1907. Effects of a late spring frost in the Southwest. Forestry and Irrig. **13**: 485-492.
5. PLAGNAT, F. 1950. Le gui du sapin. Ann. de l'Ecole Natl. des Eaux et Forets **12**: 155-231.
6. PLAGNAT, F. 1955. Le gui du sapin en France. Jour. d'Agr. Trop. et de Bot. Appl. **11**: 87-94.
7. SCHNECK, J. 1884. Notes on Phoradendron flavescens, Nutt. II. Bot. Gaz. **9**: 101-103.

USE OF SHADE TENT AND INSECTICIDES IN STUDIES OF VIRUS SPREAD [1]

Paul E. Waggoner and James B. Kring

SUMMARY

The number of green peach aphids on potato plants was greater under a tobacco shade tent than in the open field. The greater number of aphids was accompanied by greater spread of potato virus Y from infected source plants. Wettable powder formulations of DDT and Dilan used at rates to control chewing insects did not prevent aphid infestation. In fact, use of Dilan and Dilan plus DDT resulted in increased aphid populations in the field. When control of aphids was desired, sprays of malathion wettable powder effectively controlled them. Spread of potato virus Y was not increased by a spray blast of DDT directed from diseased plants to healthy ones.

Studies of spread of viruses in open fields often fail to yield conclusive results because of a lack of aphid vectors. This may result from environmental conditions unfavorable to aphid multiplication or movement or from application of insecticides to control chewing insects. Methods for improving the environment for aphids, for controlling chewing insects without injury to aphids, and for increasing movement of aphids have been tested in the experiment described in this paper.

A sheltered environment that should be more favorable than the open field to aphids is provided by shade tents of the type used for growing cigar wrapper tobacco (1). Additional protection may be given to developing aphid populations by the exclusion by the shade cloth of parasites and predators. This reasoning is borne out by the observation that aphid infestations appear earlier and more frequently in shade-grown than in field-grown tobacco in the Connecticut River Valley.

Control of chewing insects without injury to aphids might be obtained by sprays of DDT (1:1:1-trichloro-2:2-di(*p*-chlorophenyl) ethane). Both decrease and increase of aphid numbers have been reported where it was used to protect plants. This disagreement rests primarily on the difference in effectiveness of DDT against different species of aphids (2,4). It also rests on the difference in effectiveness of various formulations (4,6). For example, increase of aphid populations is more common following the use of dusts and wettable powder formulations than it is following use of emulsifiable concentrates.

Rapid increase of aphid populations also is reported where the insecticide Dilan (1 part 2-nitro-1,1-bis(*p*-chlorophenyl) propane and 2 parts 2-nitro-1,1-bis(*p*-chlorophenyl) butane) was used to protect plants from chewing insects (3, 5). Increase in aphid numbers following use of Dilan may result from an effect of the insecticide on predators and not on the aphid; however, field experience has not yet demonstrated this.

No insecticide is completely effective; therefore, aphids might be air borne by a spray blast. In this way the spread of a virus might be increased by spraying.

METHODS.—Katahdin potatoes (*Solanum tuberosum* L.) were planted at 1-ft. intervals in rows in the open field and in a shade tent. One hill of Green Mountain tubers infected with potato virus Y was planted at the centers of some rows.

Three fields within 1000 ft. of one another were used for testing the effect of sprays and of shelter upon aphids and upon virus spread. The first field of 9 rows was under a tobacco shade tent. The plants all received a spray of 4.5 lb. of 25 per cent wettable powder Dilan in 100 gal. of water on June 13, July 8, and July 15. Six of the rows received a spray of 2 lb. of 50 per cent wettable powder DDT in 100 gal. of water on June 27, June 29, July 5, July 14, July 19, July 29, and August 8. These sprays were applied by a spray jet directed through the source plant and toward the healthy plants. The spraying was then

[1] Accepted for publication May 16, 1956.

TABLE 1.—*Aphid counts on Katahdin potato plants grown in open field and in a shade tent and sprayed with various insecticides*

Plot	Insecticide	No. on 2 stems from each of 18 plants, July 11, 1955		No. on 15 terminal leaflets from 15 plants, July 29, 1955	
		M. persicae	*Macro. solanifolii*	*M. persicae*	*Macro. solanifolii*
Tent	Dilan	2109	29	206	0
	Dilan + DDT	2981	26	130	0
	Dilan + DDT + Malathion			1	0
Open	Dilan	24	22		
	Dilan + DDT	292	80		
	None	0	0	0	0

continued along the row to the end. Finally the operation was repeated from the same source plant but in the opposite direction. Three of the rows that had been sprayed with Dilan and DDT also were sprayed with 2 lb. of 25 per cent wettable powder malathion (S-1:2-bis(ethoxycarbonyl)ethyl-0,0-dimethyl dithiophosphate) in 100 gal. of water on July 11, 19, and 29.

The second field was unsheltered. The rows received the same treatments as those in the shade tent. The third field was unsheltered and received no sprays.

The numbers of aphids were estimated on July 11 from counts by species of all aphids found on 2 stems from each of 18 hills located at 1-yard intervals in each plot. On July 29, aphid populations were again estimated in the untreated, unsheltered field and in the shade tent from counts of those found on 15 uniformly selected terminal leaflets on 15 separate plants from each plot.

On August 18, a tuber was dug from each Katahdin hill in the rows containing an infected Green Mountain plant in the center. These tubers were indexed in the greenhouse for virus Y by visual observation of the plants grown from them.

RESULTS AND CONCLUSIONS.—The principal aphid species present was the green peach aphid, *Myzus persicae* (Sulzer) (Table 1). Relatively small numbers of the potato aphid, *Macrosiphum solanifolii* (Ashmead) (= *M. euphoribae* (Thomas)), were found.

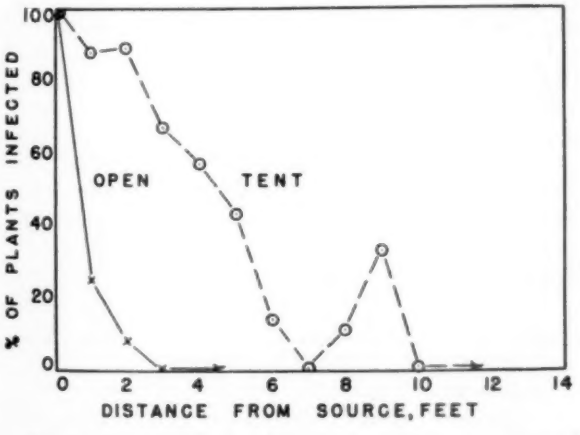


FIG. 1.—Percentage of infected plants at various distances from source plants infected by virus Y. "Open" refers to plants grown in an open field and "tent" to adjacent plants grown in a shade tent. Each point is the mean of 6 rows.

Three conclusions pertinent to the increase of aphid populations in virus experiments can be drawn. First, the shelter of the shade tent was a more favorable environment than was the open field. Second, use of Dilan resulted in increased populations in the open field; still greater increases resulted when DDT was added to the Dilan. In the shade tent, the addition of DDT to Dilan had no consistent effect on aphid populations. Finally, malathion controlled aphids and permitted vines to live until harvest in the tent. The adjacent vines in the shade tent that were treated with Dilan or Dilan plus DDT were killed by aphids in early August.

Source of virus were present only in the rows sprayed with Dilan or with Dilan plus DDT. The virus was spread along these rows. No difference in spread due to directing a spray of DDT through the source plants was evident; however, spread was greater in the tent than in the open field (Fig. 1). These results emphasize 2 things: 1) shelter increased the spread of aphid-borne virus or, conversely, an exposed location limited its spread; and 2) sprays, such as those containing DDT, directed from the virus source toward healthy plants did not cause a detectable increase in spread.

DEPARTMENTS OF CLIMATOLOGY AND ENTOMOLOGY
THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. ANDERSON, P. J. 1952. Growing tobacco in Connecticut. Conn. Agr. Exp. Sta. Bul. 564: 91-94.
2. GYRISKO, G. G., G. P. WENE, AND W. A. RAWLINS. 1946. DDT to control potato aphids. Jour. Econ. Ent. **39**: 205-208.
3. KRING, J. B. 1955. Control of DDT-resistant potato flea beetles. Conn. Agr. Exp. Sta. Circ. 193, 23 p.
4. SYLVESTER, E. S. 1949. Aphid control experiments on potatoes in California—with special reference to the selective action of DDT dusts. Jour. Econ. Ent. **42**: 766-769.
5. WHEATLEY, G. A. 1955. Dust insecticides for the control of the imported cabbage worm. Conn. Agr. Exp. Sta. Bul. 590, 15 p.
6. WOODRUFF, NANCY. 1951. Control of cutworms and aphids on tobacco. Jour. Econ. Ent. **44**: 322-324.

NEW RACES OF COLLETOTRICHUM LINDEMUTHIANUM IN MEXICO[1]

William D. Yerkes, Jr.,[2] and Moisés Teliz Ortiz[3]

SUMMARY

The susceptibility of previously resistant bean varieties to *Colletotrichum lindemuthianum* in Mexico suggested that several new physiologic races exist in that country. When 5 Mexican bean varieties were used as differentials in addition to the standard United States differentials, the presence of race beta and of 10 new Mexican races was demonstrated. The presence of resistance factors in Mexican bean lines to these races demonstrates the practicability of producing by hybridization new varieties resistant to anthracnose.

Since the studies made by Melendez[4] on the susceptibility of Mexican bean varieties to races alpha, beta, and gamma of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib.,[5] little additional work has been done on this problem in Mexico. New anthracnose-resistant varieties of field beans produced by pure line selection from native collections have been released, but these varieties are now susceptible, indicating that there are several new races of *C. lindemuthianum* in Mexico. Thus it became necessary to determine which races were present and which varieties carried resistance factors to these races.

MATERIALS AND METHODS.—From 1953 to 1955, over 30 collections of anthracnose-affected material were obtained from the principal bean-producing areas of the Central Plateau and the Bajío (West-Central Plains). The collections included infected seed and pods and leaves with veinal necrosis. Isolations were made at the Office of Special Studies laboratories at Chapingo, Mexico. Initial isolations were made on sterilized potato-dextrose agar; later, cultures were transferred to agar slants each containing a sterilized bean pod imbedded in the agar. From these isolates, single-spore cultures were obtained by dilution technique and subsequent transfer to tubes of agar containing sterilized bean pods. These cultures were incubated at 22°C until abundant spore production was obtained and then stored at 5°C.

Inoculation.—Inoculum was prepared by suspending the spores from freshly sporulating bean-pod cultures in sterile water. Twelve plants of each of 18 bean varieties, with fully expanded primary leaves, were sprayed with the conidial suspension to determine the variety reaction to the different isolates. The inoculated plants were placed in a moist chamber for 24 hours and then incubated on greenhouse benches at approximately 20°C.

Disease readings.—The readings of infection grade were taken 12 days after inoculation. The scale of disease severity was essentially that used by Melendez[4]. Five infection grades were 0, immune; 1, very light infection; 2, moderate infection; 3, severe infection; and 4, very severe infection.

Grades 0, 1, and 2 were considered as indicating resistance and 3 and 4 as indicating susceptibility. These grades are shown in Figure 1. There was 1 type of reaction that was difficult to assess. This type appeared as a leaf spotting, in which characteristic veinal lesions did not develop. It was interpreted as a resistant type of reaction (Fig. 2). Another atypical reaction was one that appeared within 5 days of inoculation as pale green chlorotic areas, usually involving most of the leaf lamina. Normal veinal lesions appeared after 9 days, at which time the interveinal areas had become pale green papery lesions, completely dry. This reaction was interpreted as one of extreme susceptibility and was commonly observed in certain varieties regardless of the source of inoculum (Fig. 3). The readings on this type of reaction were taken on the veinal lesions. Several inoculation series were repeated to check inoculation technique.

RESULTS.—From the readings taken on Michelite, Dark Red Kidney, and Perry Marrow varieties, standard anthracnose differentials, it was evident that new races were involved. There were 3 reaction types hitherto unreported on these differential varieties (Table 1).

When isolates from the Mexican anthracnose collections were placed on a number of Mexican bean varieties, it was found that the Mexican I, II, and III groups could be further differentiated into a number of races.

TABLE 1.—*Reactions of standard differential bean varieties to 3 Mexican groups of C. lindemuthianum, as compared with races alpha, beta and gamma*

Differential varieties	Reaction[a] to indicated race or group					
				Mexican groups		
	Alpha	Beta	Gamma	I	II	III
Michelite	S	R	R	R	S	R
Dark Red Kidney	R	S	S	R	S	R
Perry Marrow	R	R	S	R	R	S

[a] S indicates susceptibility and R resistance.

[1] Accepted for publication June 1, 1956. Paper No. 47, Agricultural Journal Series of The Rockefeller Foundation.

[2] Assistant Plant Pathologist, Rockefeller Foundation Agricultural Program in Mexico.

[3] Ingeniero Agrónomo, Office of Special Studies of the Mexican Ministry of Agriculture.

[4] Meléndez de la Garza, Maria de los Angeles. 1951. Reacción de frijol en México a tres razas de Colletotrichum lindemuthianum. [Mex.] Sec. de Agr. y Ganad. Ofic. de Estud. Especiales. Fol. Téc. 9, 29 p.

[5] Recent investigations by Dr. J. A. Stevenson indicate that Scribner placed the bean anthracnose organism in *Colletotrichum* before Briosi and Cavera, in 1888.

TABLE 2.—*The reactions of United States and Mexican differential bean varieties to 10 Mexican races of C. lindemuthianum as compared with those to races alpha, beta, and gamma*

Differential	Reaction to indicated race[a]												
				Group I						Group II	Group III		
	Alpha	Beta	Gamma	MA-1	MA-2	MA-3	MA-4	MA-5	MA-6	MA-7	MA-8	MA-9	MA-10
Michelite	S	R	R	R	R	R	R	R	R	S	R	R	R
Dark Red Kidney	R	S	S	R	R	R	R	R	R	S	R	R	R
Perry Marrow	R	R	S	R	R	R	R	R	R	R	S	S	S
Negro 150	S	R	R	S	S	R	R	R	R	R	S	R	S
Negro 152	S	R	R	S	S	S	S	R	R	R	S	S	S
Amarillo 155	S	R	R	S	S	S	S	S	S	R	R	R	S
Bayo 164	S	R	R	S	R	R	S	R	S	R	R	R	R
Canario 101	S	S	S	R	R	R	R	R	R	S	R	R	R
No. of collections	1	2[b]	1	8	9	3	1	1	1	1	1	1	1

[a] S indicates susceptibility and R resistance.
[b] One Mexican collection, 1 Cornell strain culture.

Five Mexican varieties were selected as differentials from among 18 lines originally tested. These were Negro 150, Negro 152, Amarillo 155, Bayo 164, and Canario 101. The lines selected have been pure-lined for at least 6 generations and were homozygous in their anthracnose reactions.

A number of other lines were discarded as differentials because of a lack of homozygosity or similarity in reaction to the above varieties. The results of these inoculations compared with standard cultures of alpha, beta, and gamma races (Cornell strains) demonstrated the existence in Mexico of race beta and of 10 new races MA-1 through MA-10. (Table 2). All of the Mexican types except Canario 101 were highly resistant to races beta and gamma. This is in general agreement with the observation of Melendez,[4] who stated that 90 per cent of 181 Mexican varieties tested were highly resistant to these races.

If enough differentials are used, all of the collections can be separated as distinct entities, but it is of questionable value to delimit new races *ad infinitum* if these races continue to be obviously closely related to those previously described. The value of this is to be questioned also with a view to the nature of the differential varieties involved. According to Freytag's study of bean races in Mexico and Guatemala,[6] the black (negro), bayo, and Canario types are distinct bean races. There is more or less uniformity in the reactions of variant races of Mexican beans to specific races of *C. lindemuthianum*. On this basis it seemed reasonable to use only lines from the distinct races as differentials, although a yellow type (Amarillo 155) falling in the bayo race was included because it separated race MA-10 from MA-8.

Schreiber[7] described 34 new races of the bean anthracnose pathogen from observations on 53 collections in Germany but found that they all fell generally into the alpha, beta, or gamma groups. Unfortunately, he used a severity index that is difficult to evaluate. Following inoculation of 20 plants of each bean variety with isolates from each collection of *C. lindemuthianum*, he described 5 grades of reaction varying from immune to destroyed; however, he based his intermediate classes on number of plants killed as well as on size of lesions. If plants were killed, they were obviously destroyed, so that his scale is not as much an indication of disease severity as it is an indication of variation in the bean varieties used. He based his 34 races on the 5 degrees of infection of 30 differential varieties. Undoubtedly this number of races could have been greatly reduced if only 2 grades of infection, resistant and susceptible, and fewer differentials had been used.

DISCUSSION.—Vavilov[8] and others are in general agreement that the centers of origin of *Phaseolus vulgaris* and *P. coccineus* (*multiflorus*) were in the Mexico-Guatemala plateau. Genetic segregants and mutants were selected and distributed by natural forces and indigenous populations in different directions from these centers.

It is not unreasonable to postulate a parallel development and spread of bean pathogens, including *C. lindemuthianum*, during this time. This is one possible explanation of the existence of the numerous races present.

Throughout Mexico we find a wide variation in the types (races) of cultivated and wild beans. For the most part the cultivated varieties are types of *P. vulgaris*, but intervention by *P. coccineus* can be distinguished in many varieties. Thus a great number of widely distributed genotypes are present in Mexico.

As Andrus and Wade[9] pointed out, there obviously is a close correlation between the genotypic varieties that occur in a host species and the number of races of its pathogens. Since the greatest known genotypic range of *P. vulgaris* probably exists in Mexico, it would follow that a wide variation in pathogenic races

[6] Freytag, G. F. Unpublished data.
[7] Schreiber, F. 1932. Resistenz züchtung bei Phaseolus vulgaris. Phytopath. Ztschr. 4: 415-454.
[8] Vavilov, N. I. 1949-1950. The origin, variation, immunity and breeding of cultivated plants [Selected writings translated by K. S. Chester]. Chronica Botanica 13: 1-364.
[9] Andrus, C. F., and B. L. Wade. 1942. The factorial interpretation of anthracnose resistance in beans. U.S. Dept. Agr. Tech. Bul. 810, 29 p.

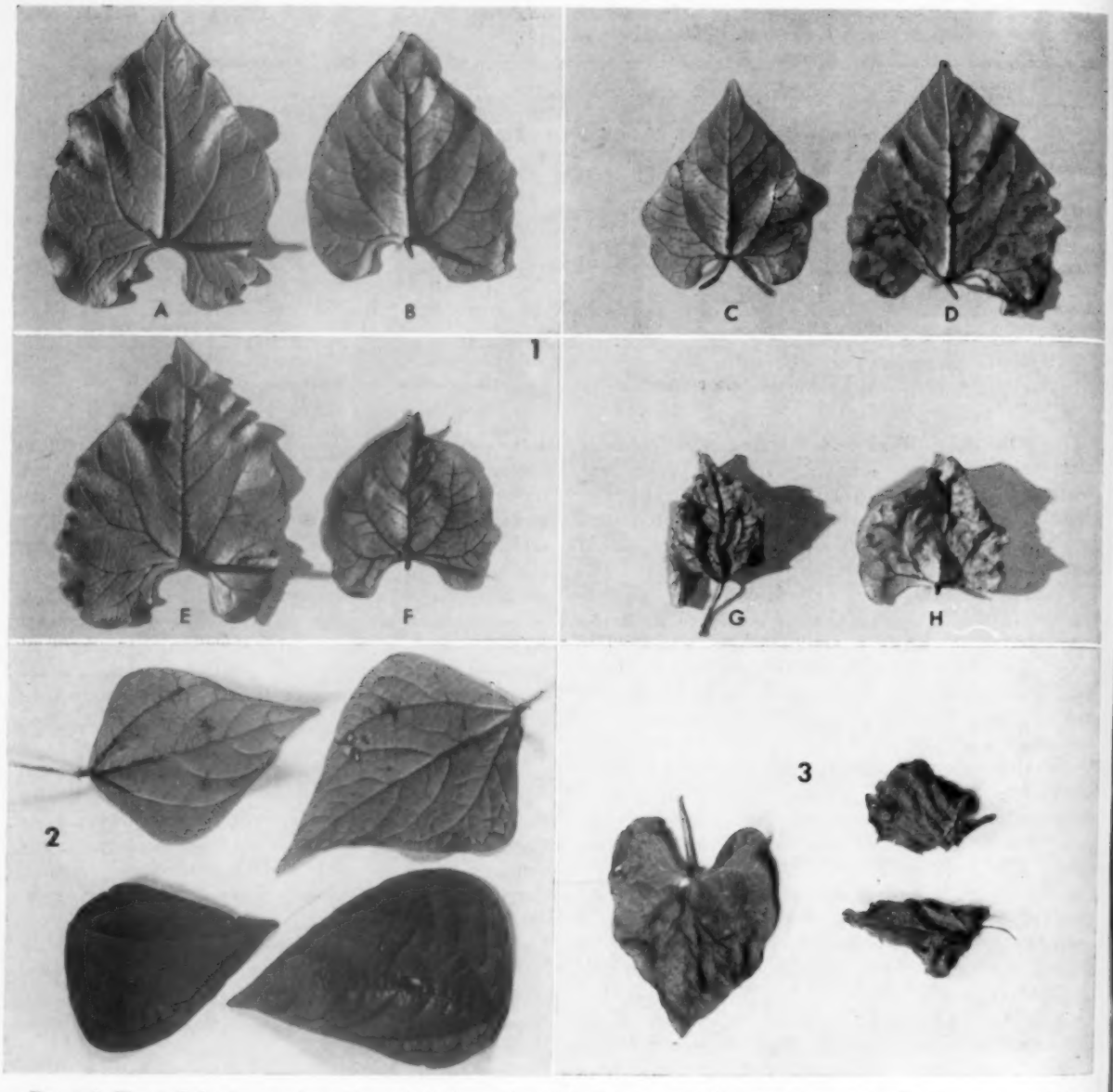


FIG. 1–3. Fig. 1. Infection grades: A) grade 0, immunity; B) grade 1, very light infection; C) grade 2, moderate infection; D) grade 3, severe infection; E,F) grade 4, very severe infection; G) very severe infection with mesophyllic chlorosis; H) severe infection with mesophyllic infection.—Fig. 2. Leaf-spotting type of reaction.—Fig. 3. General leaf chlorosis without typical anthracnose lesions.

of *C. lindemuthianum* could be expected.

Fields of "local variety" beans in Mexico are a mixture of many genotypes, usually similar only in seed color. There are also diverse resistance-susceptibility factors present, so that with any given race of the anthracnose pathogen, only a small percentage of the plants will be affected. As improved uniform varieties are released for distribution, this field variation is lost and anthracnose can become a very serious problem. The production of disease-free seed is difficult in Mexico because of the diversity of climates, of growing seasons, and of altitudes in the various bean-producing areas. For this reason, genetic control appears to be the best final solution to the problem. From information on the races of *C. lindemuthianum* present in Mexico and on the basic sources of resistance to the common field races, a breeding program can be directed toward the development of resistant varieties.

An example of such direction is one of the crosses made by the Office of Special Studies several years ago. Amarillo 154 (Rocamex 1), a pure-line selection variety released in 1949, has almost disappeared from commercial use because of its low market value, a

TABLE 3.—*Reactions of an F_6 progeny and of the parents of that progeny to different races of Colletotrichum lindemuthianum*

Race of pathogen	Reaction[a] of indicated parent or progeny		
	Amarillo 154	Canario 101	F_6[b]
MA-1	S	R	R
MA-2	S	R	R
MA-3	S	R	R
MA-4	R	R	R
MA-5	S	R	R
MA-6	R	R	R
MA-7	R	S	R
MA-8	R	R	R
MA-9	S	R	R
MA-10	S	R	R
Alpha	S	S	S
Beta	R	S	R
Gamma	R	S	R

[a] S indicates susceptibility and R resistance.
[b] Progeny II-1-834-1-1-Ux-U.

result of color prejudice and anthracnose susceptibility. In the year it was released, this variety was crossed with Canario 101, with selection in the F_2 and subsequent generations directed toward higher yielding Canario types. At the same time, resistance to anthracnose in the field was one of the factors considered in selection. The F_5 selections that were made in 1954 were uniform enough for mass harvesting, and a seed increase program was begun in 1955. The variety will be released in 1957. These lines were free of anthracnose in 1955, one of the worst years for this disease. In view of high field-resistance, the hybrid lines were tested in the greenhouse with isolates from all collections and the results indicated incorporation of resistance factors of both parents (Table 3).

Although only 1 selection is represented and no statistical studies have been made of the various generations, these data demonstrate that genetic control of this disease is possible. That the advanced generations of selection should be carefully tested is indicated by another cross, made at the same time but back crossed to Canario in the F_3. This line behaves as Canario does in its anthracnose reactions, being susceptible to races alpha, beta, gamma, and MA-7. In the back crossing, the Canario susceptibility was reintroduced into the line.

Long-range studies of the genetics of resistance and susceptibility have been begun. Meanwhile, the immediate value of racial determination is apparent, indicating what races are present and what sources of resistance are present.

ROCKEFELLER FOUNDATION AGRICULTURAL PROGRAM
CALLE LONDRES 40
MEXICO, D. F., MEXICO

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

ANDERSON, H. W. **Diseases of fruit crops.** McGraw Hill Book Co., New York. 501 p. Price $8.50

This is a comprehensive treatment of the diseases of fruits that are grown in the temperate regions of North America. Foreign distribution is given, but diseases that occur elsewhere in the world and not in the United States or Canada are not described unless they are of major importance.

In the introductory chapter, the nature of disease is discussed briefly followed by a more detailed description of the causes of disease, methods of identification, and principles of control. In the main part of the text, the diseases are treated in their order of importance on the various crops or groups. In most cases the disease is treated under the headings history, geographic distribution, economic importance, symptoms, cause, hosts, morphology of the fungus or other cause, disease cycle, relation to environment, and control.

Recent advances are described, including those pertaining to diseases caused by viruses and by nematodes. References are made to papers of historic interest and to the more important papers on each disease.

GOODEY, T., J. B. GOODEY, AND MARY T. FRANKLIN, 1956. **The nematode parasites of plants catalogued under their hosts.** Commonwealth Agricultural Bureaux, Farham Royal, Bucks, England. 140 p. Price 27s. 6d.

The authors have revised, rearranged, and brought up to date T. Goodey's 1938 publication of the same title. The present publication is a complete list of the nematode parasites of flowering plants, ferns, mosses, liverworts, seaweeds, and fungi. It consists chiefly of an alphabetical catalogue of the scientific names of plants that have been reported, up to the end of 1954, as hosts of nematodes. New and hitherto unpublished records are included. The name of the first recorder of each host-parasite association is given, as well as a list of references in which the material was first published.

ANTIBIOTICS AGAINST PLANT DISEASE. I. LABORATORY AND GREENHOUSE SURVEY[1]

Thomas G. Pridham, Lloyd A. Lindenfelser, Odette L. Shotwell, Frank H. Stodola, Robert G. Benedict, Colette Foley, Richard W. Jackson,[2] William J. Zaumeyer, William H. Preston, Jr., and John W. Mitchell

SUMMARY

Results of an extensive survey of microorganisms, principally strains of *Streptomyces*, for antibiotics effective against plant disease are presented, with an account of the methods used. About 500 pure cultures of *Streptomyces* were subjected to primary screening by 3 methods against a minimum of 12 phytopathogenic bacteria and fungi. Based on their antimicrobial spectra, some 200 strains were selected as warranting further study and were grown in a variety of media in shaken-flask fermentations. The culture filtrates were tested for antibiotic activity by paper-disc assay against 9 phytopathogenic bacteria and fungi. Fifty-two of the 200 strains show promise. Culture filtrates from 8 strains as well as 2 substances isolated from the mycelia of different strains have been tested thus far in the greenhouse against 2–10 plant diseases. Nine of the 10 products show broad antifungal spectra in laboratory tests and activity against at least 1 disease in greenhouse tests. Phytotoxic effects were observed with some of the test materials.

For the past several years the applications of antibiotics in agriculture have been rapidly expanding. The use of streptomycin and cycloheximide (Actidione) in the practical control of certain bacterial and fungal diseases of plants is now well known, and commercial preparations of these antibiotics are on the market. Several recent review articles (1, 2, 5, 8, 9) indicate the widespread interest in the field of agricultural antibiotics.

The Northern Utilization Research Branch has been searching for new antibiotics since completion of its work on penicillin in 1945, and about 2 years ago, in cooperation with the Horticultural Crops Research Branch, focused its interest on antibiotics that would be of value against plant diseases. The laboratory screening methods developed for this purpose are quite similar to those used elsewhere except that more emphasis is placed on the testing of isolates for antifungal activity. Primary screening, involving 3 techniques, is conducted with pure cultures of *Streptomyces* species. These cultures consist of fresh soil isolates, of named cultures, and of previously discovered antibiotic-producing strains in the NRRL culture collection. In the secondary screening phase, all strains retained from the primary screening are grown in various liquid media and tested for their ability to produce antagonistic factors in shaken-flask fermentations. Strains retained from the secondary screen are then subjected to more detailed study in shaken-flasks to establish optimal conditions for production of active factors. When these conditions are determined, active culture filtrates are sent to the Horticultural Crops Research Branch at Beltsville where tests are run with greenhouse-grown plants infected with any 1 of 10 selected plant pathogens. Strains that continue to show promise are subjected to preliminary chemical screening to obtain information on the stability of the active factors and on methods for their isolation. These studies include determination of the stability of culture filtrates to heating and to standing at room temperature at pH 3, 7, and 9, and determination of the possible use of common solvents or ion-exchange resins for isolation.

METHODS.—The choice of suitable test organisms in a screening program for antibiotics effective against plant diseases is of considerable importance in view of the many diseases of plants caused by bacteria and fungi. We attempted, therefore, in our initial screening, to employ the widest variety of test organisms possible. Our screening microorganisms include representative causative agents of galls, wilts, blights, rots, and smuts. Every bacterial genus that contains species of known phytopathogens is represented in both the primary and secondary screening. Since 5 of these genera are Gram-negative, the antibacterial screening is, for the most part, selective for isolates with activity against Gram-negative organisms. In the antifungal screening program, representatives of each of the major groups of fungi are included, i.e. Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti. In the selection of the test fungi, no particular emphasis was placed on any specific disease. Unless otherwise indicated, cultures of test organisms used were those bearing the NRRL numbers indicated the first time the species is mentioned.

Primary screening.—Antibacterial screening was carried out by the 'spectrum-plate' method. *Streptomyces* strains were streaked across the surface of M-1

[1] Accepted for publication June 11, 1956.

Presented in part before the 13th annual meeting of the Potomac Division of the American Phytopathological Society at the Plant Industry Station, Beltsville, Maryland, March 1, 1956.

The authors are indebted to Dr. C. W. Hesseltine, In Charge, Culture Collection Unit, Fermentation Section, Northern Utilization Research Branch, for many of the *Streptomyces* isolates screened in the survey.

[2] Authors preceding are from the Northern Utilization Research Branch, those following from the Horticultural Crops Research Branch, of the U. S. Department of Agriculture.

agar medium[3] in Petri plates and incubated for 4 days at 28°–30° C. The phytopathogenic bacteria were then streaked at right angles to the streptomycete growth. Cultures for this purpose were grown in deep-liver medium (3), modified by decreasing the K_2HPO_4 (anhydrous) concentration to 1.0 g per liter and by adjusting the pH to 7.0 before sterilization. The seeded tubes were incubated 18–24 hours at 28°–30° C on a Gump rotary shaker[4] (200 rpm). Seeded plates were incubated an additional 2 days at 28°–30° C, and zones of inhibition were measured. Strains were retained for further testing if they inhibited at least 4 of the 6 bacteria. Bacteria used for testing were *Agrobacterium tumefaciens* (E. F. Sm. et Town.) Conn, NRRL B-36; *Corynebacterium fascians* (Tilford) Dows., NRRL B-190; *Bacterium stewartii* E. F. Sm., NRRL B-195; *Erwinia carotovora* (L. R. Jones) Holland, NRRL B-204; *Pseudomonas phaseolicola* (Burk.) Dows., NRRL B-845; and *Xanthomonas phaseoli* (E. F. Sm.) Dows., NRRL B-1460.

With the exception of *X. phaseoli*, all bacterial cultures were maintained at 28°–30° C on deep-liver agar slants (deep-liver broth prepared as a solid medium by the addition of 1.5 per cent agar). *Xanthomonas phaseoli* was maintained on deep-liver agar modified by elimination of the K_2HPO_4, by increasing the agar and glucose concentrations to 20 g per l, and by adding 10 g $CaCO_3$ per l. No pH adjustment was necessary in preparing this medium.

The antifungal screening was conducted as follows. In preliminary studies, a number of Streptomycetes were examined for antifungal activity by a cut-plug technique. *Streptomyces* strains were spot-inoculated on the surface of M-1 agar and incubated for 4 days at 28°–30° C. Plugs were then cut from the agar at the edges of the colonies with a sterilized 8-mm cork borer and transferred aseptically to the surface of a synthetic agar medium (MSA)[5] seeded with selected fungi and further incubated under conditions suitable for the growth of each fungus. The diameters of zones of inhibition were then determined. Fifty ml of medium seeded with a selected fungus was pipetted into 4 × 11-in. assay plates. Forty-eight cut-plugs were placed on the surface of the seeded medium in each plate. Strains of fungi used with the cut-plug screening technique were *Trichoderma viride* Pers. ex Fries, NRRL 1700; *Mucor ramannianus* Moel., NRRL 1839; *Cladosporium herbarum* (Pers.) ex Lk., NRRL 2175; *Ustilago zeae* (Beckm.) Ung., NRRL 2321; and *Ophiostoma* (*Ceratostomella*) *ulmi* (Buis.) Nannf., NRRL 2356. Plates for the *Trichoderma* and *Mucor* species were incubated for 20 hours at 30° C and those for the *Cladosporium*, *Ustilago*, and *Ceratostomella* species for 48 hours at 25° C. The MSA medium gave good growth with all the fungi except *C. ulmi*. Supplementation of the medium with 1 per cent (v/v) corn steep liquor allowed good growth of this organism.

Inocula for preparation of the plates were prepared by cultivating the fungi on MSA slants at 25° C until abundant sporulation had occurred. The slant surfaces were then flooded with physiological salt solution and the spores, as well as some mycelial fragments, were dislodged by scraping of the surface of the growth with a sterilized loop. The spore and mycelial masses were broken up in a Waring Blendor and filtered through sterilized cotton to remove large fragments. The resultant suspensions were then diluted and added to sterile, cooled media in appropriate amounts prior to the pouring of plates.

Somewhat later, a "spray-plate" technique similar to that described by Stessel, Leben, and Keitt (6) was employed to rescreen some of the strains that had been found promising by the cut-plug technique, as well as to screen additional strains. *Streptomyces* strains were spot-inoculated on the surface of M-1 agar and incubated for 4 days at 28°–30° C. Inocula for spraying were prepared by stationary culture of the fungi in hay-infusion broth (7, p. 35) for 7–14 days at 25° C. The resultant growth was agitated with sterilized sand and filtered through sterilized cotton. Spore suspensions were then diluted with sterile water to give the optimum number of spores per ml. Each plate, containing growing streptomycete colonies, was sprayed for periods up to 10 seconds by means of an apparatus equipped with a No. 154 DeVilbiss atomizer. Sprayed plates were incubated for 3 days at 25° C, and zones of inhibition were measured. The following fungi were employed singly and in mixture in this study: *Trichoderma viride; Helminthosporium* sp. (Link) Sacc., NRRL 1679; *Mucor ramannianus; Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. et Hans., NRRL 1985; *Cladosporium herbarum; Gibberella fujikuroi* (Saw.) Wr., NRRL 2278; *Ustilago zeae;* and *Ceratostomella ulmi.*

On the basis of broad spectra, strains were retained from the primary antifungal cut-plug screening. Other strains were retained from the primary antifungal spray-plate screening on the basis of activity exhibited against each of the test fungi and their combinations.

Results obtained thus far indicate that the cut-plug technique eliminates more isolates than does the spray-plate method. Both techniques have disadvantages. The cut-plug method restricts the number of test fungi

[3] Mixture I: dehydrated corn steep liquor, 10 g; NaCl, 5 g; distilled water, 500 ml (this portion of the medium was adjusted to pH 7.0 with 1 N NaOH). Mixture II: commercial glucose, 10 g; agar, 20 g; distilled water, 500 ml. The 2 mixtures were sterilized separately for 25 minutes at 121.5°C, mixed, cooled to 45°–50°C, and poured into plates.

[4] Mention of a commercial product does not indicate that it is endorsed by the Department of Agriculture or preferred to other products of similar specifications.

[5] Per liter: asparagine, 2 g; $K_2HPO_4 \cdot 3H_2O$, 0.43 g; KH_2PO_4 (anhydrous), 0.07 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; thiamine-HCl, 0.5 mg; commercial glucose, 40 g; agar, 15 g. The pH was not adjusted. The medium was sterilized for 20 minutes at 121.5°C and cooled to 45°–50°C prior to pouring into plates.

to 1 strain per plate and involves extra manipulation in the preparation of the plugs and their transfer to seeded plates. The spray-plate method requires careful standardization of inocula so that both members of a particular combination will grow on the plates. The method usually fails to give specific information regarding the activity of an antagonist against a particular fungus—information that may be of importance in the elimination of known antibiotic producers. The spray-plate method may be of real value, however, in the screening of soil samples for initial selection of antagonists with possible broad-spectrum activity. Studies in progress indicate that the spectrum-plate method of screening, usually applied to antibacterial screening, may also be used with fungi, thereby facilitating the screening of large numbers of isolates against many fungi. At the same time a measure of activity against each test fungus is obtained.

Secondary screening.—All strains retained from the primary screening were subjected to intensive study in shaken-flask experiments with a variety of media. Shaken-flask media were prepared in 100-ml quantities in 500-ml Erlenmeyer flasks and sterilized for 30 minutes at 121.5° C. Two runs were made with each isolate. In the first run, each isolate was cultivated in 5 different media and the filtrates assayed by the agar diffusion technique with 12.5-mm filter paper discs. The 5 media used were 1) glucose basal[6] + mineral salts + 1 per cent soybean meal, 2) glucose basal + mineral salts + 1 per cent peanut meal, 3) glucose basal + mineral salts + 1 per cent dried distiller's solubles, 4) glucose basal + mineral salts + 2 per cent corn steep liquor (v/v), and 5) distilled water with 1 per cent commercial glucose + 1 per cent soybean meal.

For the second run, each isolate was further tested in the best medium selected from the first run, modified by replacing in turn the commercial glucose with sucrose, hydrol,[7] D-mannitol, starch, lactose, and glycerol, each at a 1 per cent level.

Inocula for the shaken-flask experiments were prepared by cultivating each *Streptomyces* isolate in Bennett's broth (4) on a rotary shaker for 48 hours at 28°–30° C. Each flask of fermentation medium was seeded with 1 per cent (v/v) of the resultant broth culture. Seeded flasks were incubated on a rotary shaker at 28°–30° C for periods up to 120 hours. At 72, 96, and 120 hours, samples were removed aseptically, filtered through coarse sintered glass, and the filtrates assayed against 11 test organisms by the paper-disc assay method. The possible production of heat-stable mycelial factors was investigated also; 1 set of whole cultures was adjusted to pH 7.0, autoclaved for 30 minutes at 121.5° C, and the resultant liquor assayed. The strains employed were *Agrobacterium tumefaciens; Corynebacterium fascians; Bacterium stewartii; Erwinia carotovora; Pseudomonas phaseolicola; Xanthomonas phaseoli; Trichoderma viride; Mucor ramannianus; Ustilago zeae; Alternaria solani* (Ell. et Martin) L. R. Jones et Grout., NRRL 2325; and *Botrytis allii* Munn, NRRL 2502.

To determine the effects of culture filtrates on the bacteria, paper-disc assays and M-7 agar medium[8] were employed. Twenty-four-hour deep-liver broth cultures of the organisms were diluted with physiological salt solution to give a colorimeter reading of 35 (Lumetron Photoelectric Colorimeter Mod. 400-A red filter, 650 mμ, distilled water standard). The standardized inoculum was added to melted and cooled M-7 agar (1 per cent agar concentration) in concentrations previously determined as optimal. Twenty ml of M-7 agar (2 per cent agar concentration) was poured into each Petri plate to form a basal layer. Four ml of the seeded M-7 agar was poured over the solidified basal layer. Prepared plates were held at 5° C until used. After the paper discs saturated with the culture filtrate were placed on the surface of the seeded medium, the plates were incubated at 30° C for periods ranging from 18 to 24 hours. After suitable incubation periods, zone sizes were determined with a Fisher-Lilly antibiotic zone reader.

Antifungal assays were carried out on agar plates made with a 10-ml basal layer of MSA medium (2 per cent agar concentration) and a 10-ml seeded overlayer (1 per cent agar concentration). Inocula for these assays were prepared by cultivating the fungi on MSA slants at 25° C for periods up to 4 weeks, depending upon the time required for sporulation of each fungus. Spores of *B. allii* were obtained by cultivating the organism on hay-infusion agar slants for 4 weeks. At the end of the incubation periods, all slants were flooded with physiological salt solution, the spores were dislodged with a sterilized loop, and the suspension decanted into a sterile Waring Blendor where the clumps were broken up. The resultant suspensions were filtered through sterilized cotton and adjusted with physiological salt solution to give a colorimeter reading of 40 (red filter, 650 mμ). The volumes of standardized inocula necessary for optimal results with the fungi were determined by preliminary trials. These volumes varied from 1 to 4 per cent of the seeded-layer volume.

After the paper discs saturated with the culture filtrates were placed on the surface of the seeded media, plates containing *T. viride* or *M. ramannianus* were incubated for 24 hours at 30° C, those containing *U. zeae* or *Alternaria solani* for 48 hours at 25° C, and

[6] Per liter distilled water: NaCl, 2 g; K_2HPO_4 (anhydrous), 2 g; $MnSO_4 \cdot H_2O$, 0.04 g; $ZnSO_4 \cdot 7H_2O$, 0.0044 g; ferric tartrate, 0.005 g; commercial glucose, 10 g.

[7] A by-product obtained during the preparation of glucose by acid hydrolysis of corn starch.

[8] Mixture I: beef extract, 3 g; peptone, 5 g; NaCl, 3 g; distilled water, 500 ml (when necessary, this portion of the medium was adjusted to pH 7.0 with 1 N NaOH). Mixture II: commercial glucose, 1 g; agar, 20 g; distilled water, 500 ml. The 2 mixtures were sterilized separately for 25 minutes at 121.5°C, mixed, cooled to 45°–50°C, and poured into plates.

those containing *B. allii* for 96 hours at 30° C. The diameters of the zones of inhibition were then determined.

Our experience with disc assays for determination of the activities of unknown culture filtrates indicates a need for more research on the development of standardized antifungal assays. Zones of inhibition obtained often are not as sharply defined as are those obtained with antibacterial assays. Of the phytopathogenic bacteria used, *C. fascians* proved to be an excellent assay organism. The fungi that gave the most reproducible results were *T. viride* and *M. ramannianus;* their inhibition zones could be read with relative ease. With the exception of *U. zeae*, the other fungi were characterized by floccose growth and by inhibition zones with diffuse edges. Nevertheless, in the secondary screening phase the assays were sufficiently reliable to give the information required.

Each strain retained for further screening was subjected to intensive study in shaken-flask experiments. Attempts were made to improve the production media by supplementation with inorganic salts, etc. Additional trials were conducted to determine the optimal volumes of inocula, best agitation conditions (with reciprocal or rotary shaker), and periods of time required for peak yields. Most of these experiments were conducted with 500 ml of production medium in 2.8-liter Fernbach flasks. This larger volume furnished culture filtrate sufficient for subsequent tests with infected plants and for chemical studies.

Of the strains retained following this work, 10 have been studied in considerable detail. Solid materials obtained by chemical procedures from 2 strains in addition to culture filtrates from 8 strains have been tested in the greenhouse against 2–10 plant diseases.

Greenhouse screening.—Active culture filtrates were prepared from shaken-flask production runs in Fernbach flasks, adjusted to pH 7.0 with NaOH or HCl, and shipped under refrigeration to the Horticultural Crops Research Branch for greenhouse tests. Since they are obligate parasites, the powdery mildew and rust fungi were necessarily omitted in the laboratory screening tests. However, promising antagonists were screened against these pathogens in the greenhouse, and here, attempts were made to control rusts of beans and wheat and powdery mildews of beans and bluegrass.

In tests on the effects of culture filtrates and solid materials on the development of disease symptoms of various crop plants inoculated with different pathogens, the aboveground parts were sprayed with, or dipped into, the undiluted filtrate or 0.1 per cent aqueous solutions of the solid preparations. Detailed methods used in selection of test plants, in the culturing of pathogenic organisms, in treatment and inoculation of plants, and in provision of environmental conditions necessary for symptom development were as follows.

1) *Bean rust of Pinto beans.*—Plants of uniform size and with primary leaves expanded to ¾ final size were selected. The first trifoliate leaves were beginning to expand, but the remaining trifoliate leaves were still folded in the terminal buds. Freshly harvested spores (5 mg) of *Uromyces phaseoli* var. *typica* Arth. were mixed with approximately 0.02 ml of Tween 20 (a sorbitol derivative obtainable from The Atlas Powder Co., Wilmington, Del.), and this mixture was dispersed in 10 ml of tap water. The spore suspension was stirred rapidly and applied with a soft brush to the under surface of each primary leaf of 5 plants. The antibiotic preparations were painted on the upper surface of the leaves with the aid of a cotton swab. Inoculated, but untreated, plants were used as controls. Ten days after inoculation, the pustules were fully developed on leaves of the control plants. The severity of symptoms on the control and treated leaves was then compared, and any suppression of symptoms was noted.

2) *Stem rust of wheat (Little Club variety).*—Plants of uniform size were selected; the first leaf of each was 3–4 in. long and the second leaf just emerging from the sheath of the first. Twenty-five plants were used for each treatment. The plants were sprayed with the antibiotic preparations and after 24 hours inoculated with race 15 B of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. et E. Henn. Freshly collected spores of the organism were mixed with talc, then dusted on the moistened surfaces of the first leaf blade of each plant.[9] The plants then were placed in a moist chamber for 48 hours, after which they were kept on a greenhouse bench. Twelve days after inoculation, the number of pustules evident on the treated plants was compared with the number that had developed on untreated, inoculated controls. Any inhibition of symptoms due to the presence of antibiotics was noted.

3) *Anthracnose of lima beans (Clark's Bush variety).*—Uniform plants with primary leaves partially expanded and with the first trifoliate leaves still tightly folded in the terminal buds were selected. *Colletotrichum truncatum* (Schw.) Andrus et W. D. Moore was cultured on sterilized bean pods placed on agar in Petri dishes or on potato-dextrose agar slants. When spores had developed, they were washed into water and a spore suspension was made (10–15 ml water per pod or per slant). After the plants were sprayed with the antibiotic preparations and had dried, they were sprayed with the fungus spore suspension. One week after inoculation, the treated plants were compared with inoculated, untreated controls and any suppression of symptoms due to antibiotic was noted.

4) *Anthracnose of Pinto beans.*—Methods used were the same as for lima bean anthracnose. Plants of uniform size, with primary leaves partially expanded, were used. The first trifoliate leaves were be-

[9] Spores were furnished and inoculations made by Dr. Wm. Loegering, Cereal Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

TABLE 1.—*Inhibitory activity of Streptomyces strains against selected phytopathogenic bacteria and fungi*[a]

Test organism	NRRL No.	Method	No. of *Streptomyces* strains tested	No. of strains that produced inhibition zones with indicated diameter				
				0 mm	0-10[b] mm	10-20 mm	20-30 mm	30-50 mm
Agrobacterium tumefaciens	B-36	Spectrum	482	389	65	24	4	0
Bacterium stewartii	B-195	Spectrum	479	337	81	28	20	13
Corynebacterium fascians	B-190	Spectrum	479	170	106	121	52	30
Erwinia carotovora	B-204	Spectrum	381	337	32	11	1	0
Pseudomonas phaseolicola	B-845	Spectrum	477	327	59	56	21	14
Xanthomonas phaseoli	B-1460	Spectrum	680	490	90	72	17	11
Ceratostomella ulmi	2356	Cut-plug	320	284	14	20	2	0
Cladosporium herbarum	2175	Cut-plug	319	257	31	27	4	0
Gibberella fujikuroi	2278	Spray	66	21	26	18	1	0
Helminthosporium sp.	1679	Spray	23	1	7	14	1	0
Mucor ramannianus	1839	Cut-plug and spray	444	295	24	64	38	23
Trichoderma viride	1700	Cut-plug	320	300	17	1	2	0
Ustilago zeae	2321	Cut-plug	318	283	12	14	9	0
Fusarium oxysporum f. lycopersici + *Cladosporium herbarum*	1985 + 2175	Spray	246	88	1	93	41	23
Helminthosporium sp. + *Gibberella fujikuroi*	1679 + 2278	Spray	66	27	22	15	2	0
Helminthosporium sp. + *Trichoderma viride*	1679 + 1700	Spray	297	162	6	70	29	30
Trichoderma viride + *Gibberella fujikuroi*	1700 + 2278	Spray	164	84	14	27	29	10
Ustilago zeae + *Ceratostomella ulmi*	2321 + 2356	Spray	158	25	0	23	49	61

[a]Results obtained by the cultivation of *Streptomyces* isolates on M-1 agar.
[b]Includes those manifesting partial inhibition, i.e. partial growth.

ginning to separate from the remaining leaves, which were still tightly folded in the terminal buds. The organism was *Colletotrichum lindemuthianum* (Sacc. et Magn.) Briosi et Cav.

5) *Northern leaf blight of corn (Golden Cross Bantam variety).*—Plants that were about 12 in. tall and in the 4- to 5-leaf stage were selected. Five isolates of *Helminthosporium turcicum* Pass. were grown separately on potato-dextrose agar in Petri dishes.[10] After 1 week, each growth and attendant agar were homogenized with 200 ml of water in a Waring Blendor for 2 minutes. All 5 homogenates were mixed to form a composite inoculum. After the plants were sprayed with the antibiotic preparations and had dried, they were sprayed with the suspension containing the organism and placed in a moist chamber for 48 hours. They were then kept on a greenhouse bench. Twelve days after inoculation, the leaves were examined for symptoms of the disease and the treated plants were compared with the inoculated, untreated controls. Any suppression of symptoms due to presence of the antibiotics was noted.

6) *Downy mildew of lima beans (Clark's Bush variety).*—Plants selected were 4–6 in. tall with primary leaves about 2 in. in width; the first trifoliate leaf on each plant was beginning to unfold from the terminal bud. For the preparation of inoculum, infected petioles and stems from plants previously inoculated with *Phytophthora phaseoli* Thaxt. were stirred into 50 ml of water. The fungus was sporulating abundantly on the plant parts used. The spore suspension was sprayed on the upper and lower surfaces of the primary leaves and on the terminal buds of 25 plants. Comparable inoculated, but untreated, plants were used as controls. After the spraying and inoculation, the plants were placed immediately in a fog chamber and allowed to remain for 48 hours. They were then removed and subjected to subdued light for 1–2 days before being placed under ordinary greenhouse conditions. One week after inoculation, the

[10] The organism and inoculum were supplied by Miss Alice Robert, Cereal Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

treated plants were compared with untreated controls and any suppression of symptom development due to the presence of antibiotics was noted.

7) *Powdery mildew of beans (Black Valentine variety).*—Bean plants were selected for size and uniformity after they had developed 1 fully expanded trifoliate leaf. Leaves of 20 plants were inoculated with spores of *Erysiphe polygoni* DC. shaken from infected leaves. The freshly inoculated plants were grown in a greenhouse. After the fungus had become well established, 10 uniformly infected plants were selected; 5 of these were left untreated as controls, and leaves of the remainder were sprayed with the antibiotic preparations. The amount of mildew on the treated leaves was compared with that on the untreated controls during the following several weeks.

8) *Powdery mildew of bluegrass (Merion Bluegrass variety).*—Pots containing bluegrass sod, uniformly and heavily infected with *Erysiphe graminis* DC., were selected. These sod samples were divided into 3 groups of 5 pots each. The first group was designated as controls and left untreated. The second group was sprayed once with culture filtrate and the third group was sprayed at weekly intervals for a period of 3 weeks. The effects of treatment were noted from day to day by a comparison of the treated plants with the untreated controls.

9) *Halo blight of beans (Black Valentine variety).*—Plants of uniform size with primary leaves about ⅓ fully expanded were selected; the first trifoliate leaves were still folded within the terminal buds. Primary leaves of 5 plants were dipped into the antibiotic preparations. After they had dried, the upper surfaces of the primary leaves were inoculated by the conventional abrasion method using Carborundum (300 mesh) with *Pseudomonas phaseolicola* (Burk.) Dows. A diluted 48-hour beef-broth culture of the organism was used (1 part of culture diluted with 10 parts of water). Comparable leaves of untreated plants were inoculated in a similar manner and designated as controls. Six or 7 days after inoculation, the number of lesions produced on the under surface of the treated leaves was compared with the number on leaves of the control plants, and any suppression of symptoms due to the presence of antibiotics was noted.

10) *Common blight of Pinto beans.*—Plants of uniform size with primary leaves about ⅓ fully expanded were selected. The first trifoliate leaves of these plants were beginning to develop separately from the others, which were still folded in the terminal buds. The primary leaves of 5 plants were dipped into the antibiotic preparations, and after the leaves had dried, their upper surfaces were inoculated with *Xanthomonas phaseoli* (E. F. Sm.) Dows. by the abrasion method referred to above. A 48-hour beef-broth culture of the organism diluted 1 to 10 with water was used as inoculum. Ten days after inoculation, primary leaves of the treated plants were compared with those of the untreated controls and any suppression of symptom development by the antibiotics was noted.

Results.—In the primary screening, about 500 strains of *Streptomyces* were explored by the spectrum-plate, cut-plug, and spray-plate techniques. The results are presented in Table 1.

About 200 strains were selected. The majority of these possessed rather broad antibacterial and antifungal spectra, although some were predominantly active against bacteria and others against fungi. All 200 strains were subjected to secondary screening studies.

The results of primary screening indicate that the only Gram-positive bacterium employed, *Corynebacterium fascians*, was the test organism most sensitive to antibiotic factors produced by *Streptomyces* strains. In contrast, most of the Gram-negative bacteria were about equally resistant to the antagonists, although *Agrobacterium tumefaciens* appeared to be the most resistant of these. Some of the resistance of the Gram-negative organisms might be attributed to the slimy material secreted by some strains, notably *A. tumefaciens* and *Xanthomonas phaseoli*. Possibly the slimy secretion prevents access of the antibiotic to the cells. Such a condition of the phytopathogen in diseased plants might be a factor of considerable im-

Table 2.—*Activity of culture filtrates of selected Streptomyces as indicated by size of zones of inhibition by disc assay*

Test Organism	Diameter of inhibition zone produced by culture filtrate of indicated *Streptomyces* strain									
	F-6	F-9	F-12	F-14	F-17[a]	F-21	F-41	F-74	F-102	F-113
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Agrobacterium tumefaciens	0	0	25	0	0	0	0	0		18
Bacterium stewartii	0	0	17	20	0	0	0	0	0	0
Corynebacterium fascians	18	21	19	26	32	26	10	0	18	28
Erwinia carotovora	0	0	0	0	0	0	0	0	0	0
Pseudomonas phaseolicola	0	0	17	19	0	0	0	0	37	18
Xanthomonas phaseoli	0	0	20	24	0	0	0	0	35	0
Alternaria solani	27	30	0	21	35	32	21	20	31	0
Botrytis allii	45		Trace	28	45	48	28	20		28
Mucor ramannianus	26	26	0	23/33[b]	40	30	22	21	31	20
Trichoderma viride	50	26	0	Trace	27	24	21	18	29	20
Ustilago zeae	19	30	35	32	23	33	29	21	30	0

[a] NRRL B-1699.
[b] Complete inhibition to 23 mm and partial from 23 to 33 mm.

TABLE 3.—*Extent of control*[a] *of disease symptoms by undiluted culture filtrates and by mycelial preparations at a concentration of 1000 ppm in water*

			Control by filtrate or mycelial preparation of indicated *Streptomyces* strain								
			Filtrates							Mycelial preparations	
Disease	Host	Run	F-6	F-9	F-12	F-14	F-17[b]	F-21	F-41	F-113	F-74	F-102
Anthracnose	Lima bean	1			0	0	0		++		0	0
		2							0			
Anthracnose	Snap bean	1			0	0	++		+		0	++
		2										0
Common Blight	Bean	1			0	0						
		2										
Downy Mildew	Lima bean	1	0[c]	++[c]	0	++++	++	0[c]	0	0[c]	0	0
		2					++++		0		++	0
Halo Blight	Bean	1			++	0	0[c]		0[c]		0	0[c]
		2			+							
Northern Leaf Blight	Corn	1			0	0	0		0		0	0
		2										
Powdery Mildew	Bean	1			0	0	+++		0		0	0
		2										
Powdery Mildew	Bluegrass	1					++++					
		2										
Rust	Bean	1	+	+	++	+++	++++	+		+		++++
		2										
Stem Rust	Wheat	1			+	+++	+++					0
		2			0	0	+++					

[a] Protection, except for eradication of powdery mildew. Legend: 0 = none, + = slight, ++ = moderate, +++ = marked, ++++ = complete.
[b] NRRL B-1699.
[c] Filtrate toxic to plants.

portance in antibiotic therapy.

Little can be said at present concerning the relative susceptibility of the test fungi to antagonistic factors produced by *Streptomyces* strains. In general, our results indicate that *Trichoderma*, *Ustilago* and *Ceratostomella* species may be more resistant than species of the other genera to antibiotics produced by *Streptomyces* strains. Work with the spectrum-plate method indicates that the genus *Fusarium* (*Gibberella*) also may be highly resistant. We have not yet accumulated sufficient data to rank the 4 major groups of fungi in the order of their sensitivity to antibiotic factors. Even if marked differences do occur between groups, it is not known that use of the more resistant fungi would lead us to effective new antibiotics.

In the secondary screening, 52 strains were selected on the basis of their activities against the 11 test organisms. Many of these strains exhibit both antibacterial and antifungal activity. A few were retained because of their outstanding activity against specific organisms. Table 2 contains some representative results of paper-disc assays of culture filtrates of these strains. The remainder of the promising strains are still under test.

Of the strains screened thus far, known producers of actithiazic acid, aureothricin, hygroscopin, musarin, neomycin complex, streptothricin complex, streptomycin, vinactin, and xanthomycin have been eliminated, either in the primary or in the secondary screening stages.

The majority of the strains retained have been found to produce the greatest antibiotic activity in liquid media containing soybean meal or peanut meal, although there are a few exceptions.

Of the 10 materials tested thus far in the greenhouse, most have shown promise in controlling at least 1 of the test diseases (Table 3). It is entirely possible that some of the activities may be due to antibiotic factors that have been reported in the literature. Work directed toward the identification of the strains of organisms and of their antibiotic products is in progress.

FERMENTATION SECTION
NORTHERN UTILIZATION RESEARCH BRANCH
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
PEORIA, ILLINOIS

AND

VEGETABLE CROPS SECTION AND BASIC RESEARCH SECTION
HORTICULTURAL CROPS RESEARCH BRANCH
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. ANDERSON, H. W., AND D. GOTTLIEB. 1952. Plant disease control with antibiotics. Econ. Bot. **6**: 294-308.

2. Brian, P. W. 1954. The use of antibiotics for control of plant diseases caused by bacteria and fungi. Jour. Appl. Bact. **17**: 142-151.
3. Haynes, W. C., L. J. Wickerham, and C. W. Hesseltine. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. **3**: 361-368.
4. Jones, K. L. 1949. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. Jour. Bact. **57**: 141-145.
5. Leben, C., and G. W. Keitt. 1954. Effects of antibiotics in control of plant diseases. Agr. and Food Chem. **2**: 234-249.
6. Stessel, G. J., C. Leben, and G. W. Keitt. 1953. Screening tests designed to discover antibiotics suitable for plant disease control. Mycologia **45**: 325-334.
7. Thom, C., and K. B. Raper. 1945. A manual of the Aspergilli. Williams and Wilkins, Baltimore. 373 p.
8. Zaumeyer, W. J. 1955. Antibiotics and plant health. Agr. and Food Chem. **3**: 112-116.
9. Zaumeyer, W. J. 1956. Improving plant health with antibiotics. 1st Internatl. Conf. on the Use of Antibiotics in Agr. Natl. Acad. Sci., Natl. Res. Council, Pub. 397: 171-187.

ANTIBIOTICS AGAINST PLANT DISEASE. II. EFFECTIVE AGENTS PRODUCED BY STREPTOMYCES CINNAMOMEUS FORMA AZACOLUTA F. NOV.[1]

Thomas G. Pridham, Odette L. Shotwell, Frank H. Stodola, Lloyd A. Lindenfelser, Robert G. Benedict, and Richard W. Jackson

SUMMARY

A strain of *Streptomyces cinnamomeus* has been found to produce a mixture of antibiotics that are antagonistic to several phytopathogenic bacteria and fungi in the laboratory. This streptomycete is characterized by the whorled morphology and cinnamon-pink color of its sporulating aerial mycelium. It is considered to be a physiological form of *S. cinnamomeus* and is designated *Streptomyces cinnamomeus* forma *azacoluta* f. nov., NRRL B-1699. The mixture of antibiotics is produced when the organism is grown in a medium of tap water, soybean meal, and glucose. Tests conducted in the greenhouse show that culture filtrates are effective against rust, anthracnose, downy mildew, and powdery mildew of beans; stem rust of wheat; and powdery mildew of bluegrass. Paper chromatography has so far revealed 3 antibiotics in the mixture. Assays in the laboratory and bean rust assays performed in the greenhouse on numerous chemical fractions indicate that none of the antibiotics is alone responsible for the activity against bean rust. Potency is retained in the dried filtrate.

For about 2 years, the Northern Utilization Research Branch in cooperation with the Horticultural Crops Research Branch has surveyed microorganisms for new antibiotics effective against plant disease. Methods and preliminary results are described in the preceding paper (11). The present paper reports the results of a study of one of the more promising strains (strain F-17, now designated as NRRL B-1699), which produces the antibiotics designated as the "F-17 mixture."

During greenhouse tests, culture filtrates of this organism showed promise as a possible control agent against 6 plant diseases. Stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. et E. Henn., Race No. 15B) of Little Club wheat and rust (caused by *Uromyces phaseoli* var. *typica* Arth.) of Pinto beans were markedly inhibited. Consequently, work with this streptomycete strain was intensified. The over-all results indicate that the microorganism produces a mixture of 3 antibiotics and possibly of 4. As reported (11), these factors also may be useful in controlling anthracnose (caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Briosi et Cav.) of Pinto beans; powdery mildew (caused by *Erysiphe graminis* DC.) of Merion bluegrass; powdery mildew (caused by *Erysiphe polygoni* DC.) of Black Valentine beans; and downy mildew (caused by *Phytophthora phaseoli* Thaxt.) of Clark's Bush lima beans. One field trial against stem rust of wheat has been conducted by Dr. W. Q. Loegering, Field Crops Research Branch. Unfortunately, the favorable results obtained in greenhouse tests were not realized in this field trial.

The antibiotic mixture produced by this streptomycete has other interesting biological effects. Culture filtrates have been found to prevent growth of *Clostridium botulinum* (Van Ermengem) Holland (NRRL B-1218, N.C.A.A., 62-A) completely at dilutions up to 1:17,000 when tested by a modification of Andersen's anaerobic plate method (2). At dilutions between 1:700 and 1:800, they prevented the growth

[1] Accepted for publication June 11, 1956.
Presented in part before the 13th annual meeting of the Potomac Division of the American Phytopathological Society at the Plant Industry Station, Beltsville, Maryland, March 1, 1956.
The authors gratefully acknowledge the assistance of Dr. J. W. Mitchell, Basic Research Section, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, in carrying out the antirust assays (on bean plants) necessary for the work reported. They are also indebted to Dr. D. P. Rogers, Curator, The New York Botanical Garden, for reviewing and editing the Latin diagnoses.

of *Mycobacterium tuberculosis* var. *hominis* Lehmann et Neumann (streptomycin-resistant strain H 37 RvR, NRRL B-1300) in serial dilution assays in which the Tween-albumin medium (4) of Dubos and Middlebrook was used. When tested by the agar-streak dilution method, culture filtrates (approximately 16.9 mg solids per ml) have been found to inhibit completely the growth of *Bacillus subtilis* Cohn emend. Prazmowski (NRRL B-765), *Saccharomyces pastorianus* Hans. (NRRL Y-139), and *Trichoderma viride* Pers. ex Fries (NRRL 1700) at filtrate dilutions of 1:1000 but not at dilutions of 1:10,000. Spray-dried culture filtrates, reconstituted with distilled water, have given essentially similar results. Spectrum plate studies show that the streptomycete inhibits *Brucella bronchiseptica* (Ferry) Topley et Wilson (NRRL B-140), *Candida albicans* (Robin) Berk. (NRRL Y-477), and several Gram-positive and Gram-negative bacteria but has no appreciable activity against *Pseudomonas aeruginosa* (Schroet.) Mig. (NRRL B-23) or *Proteus vulgaris* Hauser (NRRL B-417).

CHARACTERISTICS AND TAXONOMY OF THE MICROORGANISMS.—Early in these studies it became apparent that strain NRRL B-1699 was closely related to various whorled or verticillate species of *Streptomyces*, e.g. *S. cinnamoneus* Benedict et al, *S. griseocarneus* Benedict et al, *S. netropsis* Finlay et Sobin, *S. reticuli* (Waks. et Curtis) Waks. et Henrici, *S. albireticuli* Nakazawa et al, *S. rubrireticuli* (Waks.) Waks. et al, and *S. albus* (Rossi Doria emend. Krainsky) Waks. et Henrici (thiolutin producer). Comparison of the characteristics of strain NRRL B-1699 with published descriptions and actual comparison of growing cultures by the methods of Waksman (13), of Millard and Burr (9), and of Duché (5) indicated a close relationship of this streptomycete to *S. cinnamoneus* Benedict et al (3, 7, 14). The latter organism produces the polypeptide antibiotic cinnamycin and another unidentified antibiotic. Carbon- and nitrogen-utilization tests (10) further substantiate their similarity. Aside from the production of different antibiotics as proved by paper chromatography (Table 1), the only apparent difference between *S. cinnamoneus* (NRRL B-1285) and strain NRRL B-1699 appears to be in the development, on most media, of a more deeply pigmented aerial mycelium and reverse color by the former as the cultures age.

TABLE 1.—*Paper chromatographic comparison of culture filtrates from strain NRRL B-1699 and strain NRRL B-1285*

Filtrate	R_f values of zones of inhibition of indicated organism	
	Bacillus subtilis (NRRL B-765)	*Saccharomyces pastorianus* (NRRL Y-139)
NRRL B-1699 (F-17)	0, 0.40	0.40, 0.75
NRRL B-1285	0, 0.55 (cinnamycin)	0.55 (cinnamycin)
Mixture	0, 0.40, 0.55	0.40, 0.55, 0.75

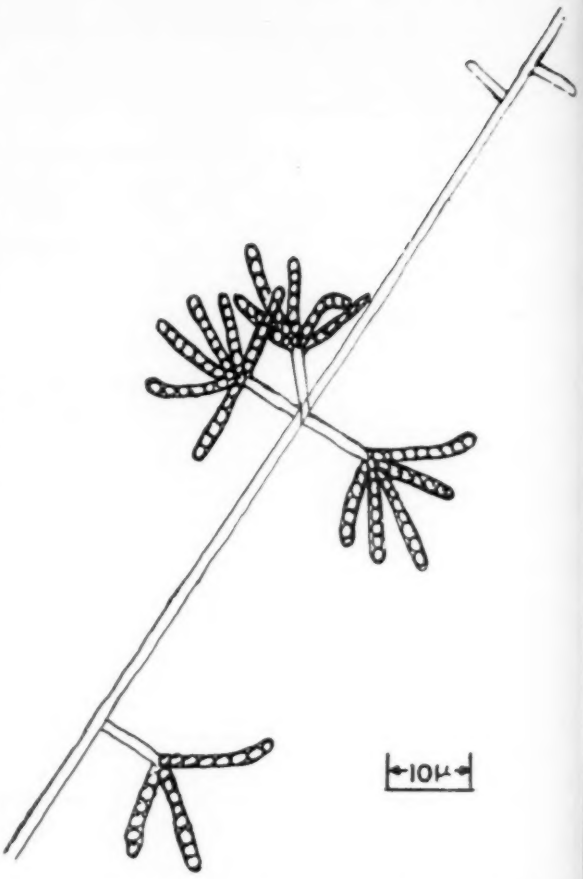


FIG. 1. Typical morphology of sporulating cultures of *Streptomyces cinnamomeus* f. *azacoluta*, NRRL B-1699.

Because of the cultural similarities of the 2 strains, the elaboration of different antibiotic factors, and the marked effect of strain NRRL B-1699 against the fungi tested, it is proposed that this strain be named *Streptomyces cinnamoneus* forma *azacoluta*. A description of this form follows an enlarged description of *S. cinnamoneus* (NRRL B-1285) which has been revised to conform to the Botanical Code. An orthographic error in the original specific epithet has been corrected in the revised description.

Streptomyces cinnamomeus Benedict, Dvonch, Shotwell, Pridham, and Lindenfelser, sp. nov.

Synonyms: *Streptomyces cinnamomeus* Benedict et al nom. nud. Benedict, R. G., W. Dvonch, O. L. Shotwell, T. G. Pridham, and L. A. Lindenfelser. Antibiotics and Chemotherapy **2**: 591. 1952; Waksman, S. A., and H. A. Lechevalier. Guide to the Classification of the Actinomycetes and their Antibiotics. Williams and Wilkins, Baltimore, p. 85-86. 1953; Dvonch, W., O. L. Shotwell, R. G. Benedict, T. G. Pridham, and L. A. Lindenfelser, Antibiotics and Chemotherapy **4**: 1140-1141. 1954.

Ilustration: Duggar, B. M., E. J. Backus, and T. H. Campbell, Fig. 12, 1954 (6).

STREPTOMYCES CINNAMOMEUS Benedict et al forma **cinnamomeus.**

Sporae catenulatae; sporophora opposita ramosa et multipliciter verticillata; rami primi 5—10 × 2 μ; rami secundarii 7–10 × 2 μ; spirae nullae; mycelium aerium in

culturis sporulantibus in coloribus subcinnameis ad puniceo-cinnameis (tinctis colorum Testaceous, Vinaceous-Tawny, Orange-Cinnamon, Cinnamon et Clay Color, denominatis a Ridgway, 1912); colore parti aversae plerumque subflavo (tinctis colorum Ochraceous-Orange, Yellow-Ocher, Primuline Yellow, Wax Yellow, Orange-Cinnamon, Cinnamon, Honey-Yellow, Olive-Ocher, et Olive-Yellow, et umbris colorarum Clay Color, et Honey-Yellow, denominatis a Ridgway, 1912); cum pigmento solubili subflavo, si adest; incrementum robustum ad 28°–30° C; in agaro cum peptono ferreo non nigrescit; in agaro synthetico ex Pridham et Gottlieb, cum D-glucoso, et *i*-inositolo crescit; in agaro cum D-fructoso languide crescit; non crescit in agaro cum L-xyloso, L-arabinoso, rhamnoso, sucroso, lactoso, raffinoso, et salicino; aliquot formae huius speciei formant antibioticum cinnamycin denominatum et antibiotica bacteriis, fermentis, et fungis adversa. Typus NRRL B-1285, 1948, ex solo ad Kanto, Saitama Prefecture, Japan, sub numero A-725 ab R. G. Benedict, lecto, in Cult. Coll. Northern Utilization Research Branch depositus.

Species epithet derived from the Latin 'cinnamōmum' meaning cinnamon and here referring to the cinnamon color of aerial mycelium of sporulating colonies.

STREPTOMYCES CINNAMOMEUS forma ***azacoluta*** f. nov. (Fig. 1)

Sporae catenulatae; sporophora opposita ramosa et multipliciter verticillata; rami primi 4.8–9.6 × 1.2–1.8 μ; rami secundarii 7.2–9.6 × 1.2–1.8 μ; spirae nullae; mycelium aerium in culturis sporulantibus in coloribus prope Shell Pink, Pale Pinkish Cinnamon, et Pale Pinkish Buff, denominatis a Ridgway, 1912; colore parti aversae prope Light Buff, Straw Yellow, Cream Color, et Chamois, denominatis a Ridgway, 1912; vulgo mycelii aerii et partis aversae colore dilutione quam *S. cinnamomei*, NRRL B-1285; cum pigmento solubili subflavo, si adest; incrementum robustum ad 28°–30° C; in agaro cum peptono ferreo non nigrescit; in agaro synthetico ex Pridham et Gottlieb, cum D-glucoso, et *i*-inositolo crescit; in agaro cum D-fructoso languide crescit; non crescit in agaro cum L-xyloso, L-arabinoso, rhamnoso, sucroso, lactoso, raffinoso, et salicino; format permistionem F-17 antibioticam cuius actio bacteriis, fermentis, et fungis adversa. Typus NRRL B-1699, 1953 ex solo pascui ad Brighton, Iowa, U. S. A., sub numero S-205 ab C. W. Hesseltine lecto et sub numero F-17 ab T. G. Pridham, in Cult. Coll. Northern Utilization Research Branch depositus.

Form epithet derived from the Greek αζα meaning "mold" and κωλυτης meaning "a hinderer." Hence "a hinderer of molds," referring to the marked antifungal activity of this form.

Aside from the production of different antibiotic factors as shown by paper chromatography, the only apparent difference between *S. cinnamomeus* f. *cinnamomeus* (NRRL B-1285) and *S. cinnamomeus* f. *azacoluta* (NRRL B-1699) appears to be the development of a more deeply pigmented aerial mycelium and reverse color by the former on most media as the cultures age.

Some important cultural characteristics of *S. cinnamomeus* f. *azacoluta* are given in Table 2 and are derived from application of general techniques outlined by Hesseltine, Benedict, and Pridham (8). Readings are based on a number of separate experiments. All cultures were incubated for 14 days at 28°–30° C.

The characteristics of *S. cinnamomeus* f. *cinnamomeus* and of *S. cinnamomeus* f. *azacoluta* summarized in Table 3 are considered to be of taxonomic significance.

METHOD OF ASSAY.—Antibiotic activity was measured by paper-disc assay both in the determination of optimal conditions for production and in the chemical studies of the F-17 mixture. The assay organisms were *Trichoderma viride* (NRRL 1700), *Saccharomyces pastorianus* (NRRL Y-139), and *Bacillus subtilis* (NRRL B-765).

Assay with Trichoderma viride.—1) *Organism and spore suspension.—T. viride* was maintained on MSA medium (11). Spores of the organism were obtained by flooding the surface of slant cultures with physio-

TABLE 2.—*Cultural characteristics and morphology of Streptomyces cinnamomeus f. azacoluta, NRRL B-1699*

Medium[b]	Amount of growth	Degree of sporulation	Color[a]		Morphology (× 100-× 400)
			Aerial mycelium and spores	Reverse	
Czapek's solution agar	Fair	Fair	Near Pale Pinkish Cinnamon	Near Light Buff	Compound whorls, no spirals
Asparagine dextrose agar	Fair	Poor	Near Pale Pinkish Buff	Near Straw Yellow	
Synthetic starch agar	Excellent	Excellent	Near Shell Pink	Near Cream Color	Compound whorls, no spirals
Carvajal's oatmeal agar	Excellent	Excellent	Near Shell Pink	Near Cream Color	Compound whorls, no spirals
Yeast extract agar	Fair	Poor	Near Pale Pinkish Buff	Near Chamois	
Tomato-paste oatmeal agar of Asheshov et al	Excellent	Excellent	Near Shell Pink	Near Cream Color	Compound whorls, no spirals
Hickey and Tresner's 'amidex' agar	Excellent	Excellent	Near Shell Pink	Near Cream Color	Compound whorls, no spirals
Cobalt[c] agar	Excellent	Excellent	Near Pale Pinkish Cinnamon	Near Cream Color	Compound whorls, no spirals

[a] Names of colors taken from Ridgway (12).
[b] For composition of media see Hesseltine et al (8).
[c] Bennett's agar supplemented with 0.002 per cent $CoCl_2 \cdot 6H_2O$.

TABLE 3.—*Comparison of Streptomyces cinnamomeus f. cinnamomeus (NRRL B-1285) and S. cinnamomeus f. azacoluta (NRRL B-1699)*

Property compared	*S. cinnamomeus* f. *cinnamomeus* (NRRL B-1285)	*S. cinnamomeus* f. *azacoluta* (NRRL B-1699)
Antibiotic	Cinnamycin and unidentified antibiotic	F-17 mixture
Morphology	Compound verticillate, no spirals	Compound verticillate, no spirals
Spore color	Cinnamon pink	Cinnamon pink
Reverse color	Buff to yellow brown	Buff to yellow
Soluble pigment	None to faint yellow	None to faint yellow
Blackening of peptone iron agar	—	—
Utilization[a] of		
L-Xylose	—	—
L-Arabinose	—	—
Rhamnose	—	—
D-Glucose	+	+
D-Fructose	⊕	⊕
Sucrose	—	—
Lactose	—	—
Raffinose	—	—
i-Inositol	+	+
Salicin	—	—
Antibiotic spectrum[b] (diameter of inhibition zone in mm)		
Bacillus subtilis, NRRL B-971	20-25	20-25
Sarcina lutea Schroet., NRRL B-1018	0	0
Escherichia coli (Mig.) Castell. et Chalmers, NRRL B-766	0	0
Candida albicans, NRRL Y-477	11-16	14-19
Mucor ramannianus Moel., NRRL 1839	16/35[c]	25/35[c]

[a] Pridham and Gottlieb's method, 10 days' incubation at 28°–30° C (— = no utilization; ⊕ = sparse utilization; + = utilization).

[b] On Pridham and Gottlieb's D-glucose synthetic agar.

[c] Diagonal indicates partial inhibition with zone to the extent of the second number; complete inhibition occurred to extent of first number.

logical salt solution and scraping the surface with a sterilized loop. Spore suspensions were adjusted to a count of 30,000,000 ± 1,000,000 spores per ml (reading of 40 with red filter 650 mμ on Lumetron colorimeter[2]) with physiological salt solution. The adjusted spore suspension was added at the rate of 1 per cent (v/v) to melted and cooled synthetic agar medium.[3]

[2] Mention of a commercial product does not indicate that it is endorsed by the Department of Agriculture or preferred to other products of similar specifications.

[3] Per liter: asparagine, 2 g; KH_2PO_4 (anhydrous), 0.78 g; Na_2HPO_4 (anhydrous), 0.63 g; NaCl, 1 g; Bacto dextrose, 10 g; pH 6.8-6.9; agar, 15 g. Sterilized for 15 minutes at 121.5° C.

2) *Preparation of assay plates.*—Assay plates with a seeded 10-ml layer were used and were prepared daily before use. Paper discs saturated with the material to be assayed were placed upon the surface of the assay agar and incubated for 16–18 hours at 30° C. The diameter of inhibition zones was determined on a Fisher-Lilly antibiotic zone reader.

Assay with Saccharomyces pastorianus.—The Actidione assay procedure (15) was used with *S. pastorianus.*

Assay with Bacillus subtilis.—1) *Organism and spore suspension.—B. subtilis* was maintained on liver-extract agar slants.[4] Spores were obtained by the method of Abbey (1). Spore suspensions adjusted to a count of 45,000,000 spores per ml were prepared in sterile physiological salt solution and added at the rate of 1 per cent (v/v) to melted and cooled MK-8[5] agar medium. Prepared and adjusted spore suspensions have remained stable for at least 1 year when stored at 3°–5° C.

2) *Preparation of assay plates.*—Assay plates with a basal agar layer and a seeded agar layer were used. The basal layer consisted of MK-8 agar (2 per cent agar concentration). Twenty ml of basal layer medium was pipetted into sterile flat-bottomed Pyrex Petri plates and allowed to solidify. The seeded layer consisted of MK-8 agar (1 per cent agar concentration) with commercial glucose omitted. (Plates may be prepared beforehand and stored for as long as 4 days at 3°–5°C). Paper discs, saturated with the material to be assayed, were placed upon the surface of the assay agar immediately after removal from storage at 3°–5° C. The assay plates were then incubated at 30° C for 16–18 hours and the diameter of inhibition zones measured on a Fisher-Lilly antibiotic zone reader.

Greenhouse assay.—A laboratory assay method for measuring antirust activity has not been developed. Attempts are being made to develop a uredospore-germination assay to measure such activity. In the present work, the greenhouse method previously described (11) was used for assaying chemical fractions for antirust activity on beans.

ANTIBIOTIC PRODUCTION IN SHAKEN-FLASKS AND

[4] Per liter: tryptone, 10 g; carrot extract, 50 ml; liver extract, 100 ml; distilled water, 850 ml. Adjusted to pH 7.0 with 1 N NaOH; 20 g agar added; steamed at 100° C for 30 minutes; 2.5 g commercial glucose added; dispensed and sterilized for 20 minutes at 121.5° C. The carrot extract was prepared by grinding 454 g of carrots, mixing the ground carrots and juice with 1 liter of distilled water, steaming at 100° C for 30 minutes, filtering through cheesecloth, shaking with Celite No. 512 filter-aid, and filtering once through a Büchner funnel before the filter-aid settled, bottling the filtrate, and sterilizing for 20 minutes at 121.5° C.

[5] Per liter: beef extract, 1.5 g; yeast extract, 3 g; peptone, 6 g; N-Z-Case (Sheffield Chemical Company, Norwich, New York), 4 g; distilled water 1000 ml; adjusted to pH 8.0 with 1 N NaOH, agar added as specified, 10 g or 20 g; dispensed and sterilized for 20-30 minutes at 121.5° C; after cooling, and before preparation of plates, 1 ml of a sterile 50 per cent solution of commercial glucose was added if specified.

TABLE 4.—*Range of antibiotic activity of S. cinnamomeus f. azacoluta, NRRL B-1699*[a]

Test organism	NRRL	Zone of inhibition	Method
		mm	
Aerobacter aerogenes (Kruse) Beijer.	B-199	16	Spectrum
Agrobacterium tumefaciens (E. F. Sm. et Town.) Conn	B-36	0	Spectrum
Bacillus subtilis	B-971	22	Spectrum
B. subtilis[b]	B-972	22	Spectrum
Bacterium cattleyae Pav.	B-835	5	Spectrum
Bacterium stewartii E. F. Sm.	B-195	11	Spectrum
Bodenheimer bacillus[c]	B-962	17	Spectrum
Brucella bronchiseptica	B-140	20	Spectrum
Corynebacterium fascians (Tilford) Dows.	B-190	15	Spectrum
Corynebacterium flaccumfaciens (Hedges) Dows.	B-729	5	Spectrum
Erwinia aroideae (Town.) Holland	B-134	0	Spectrum
Erwinia carotovora (L. R. Jones) Holland	B-204	0	Spectrum
Escherichia coli	B-766	19	Spectrum
Mycobacterium sp. Lehmann et Neumann	B-692	22	Spectrum
Proteus vulgaris	B-417	5	Spectrum
Pseudomonas aeruginosa	B-23	0	Spectrum
Pseudomonas cepacia Burk.	B-1101	0	Spectrum
Pseudomonas lachrymans (Smith et Bryan) Carsner	B-1629	0	Spectrum
Pseudomonas phaseolicola (Burk.) Dows.	B-845	0	Spectrum
Sarcina lutea	B-1018	10	Spectrum
Staphylococcus pyogenes var. *aureus* Rosenbach	B-313	10	Spectrum
Xanthomonas campestris (Pam.) Dows.	B-1459	2	Spectrum
Xanthomonas phaseoli (E. F. Sm.) Dows.	B-1460	18	Cut-plug
		2	Spectrum
Candida albicans	Y-477	35	Spectrum
Aspergillus fumigatus Fres.	1217B	6/15[d]	Spectrum
Cephalosporium sp. Corda	1866	5/20	Spectrum
Cladosporium herbarum (Pers.) Lk.	2175	12	Cut-plug
		12	Spectrum
Colletotrichum circinans (Berk.) Vogl.	2504	15/25	Spectrum
Fusarium aquaeductuum (Radl. et Rab.) Sacc.	2503	17	Spectrum
Fusarium oxysporum f. *lycopersici* (Sacc.) Snyd. et Hans.	1985	0/14	Spectrum
Gibberella fujikuroi (Saw.) Wr.	2278	12	Spray
		7/15	Spectrum
Helminthosporium sp. (Lk.) Sacc.	1679	13	Spray
Mucor ramannianus	1839	17	Cut-plug
		18	Spectrum
		30	Spray
Ophiostoma (*Ceratostomella*) *ulmi* (Buis.) Nannf.	2356	10	Cut-plug
		17	Spectrum
Penicillium notatum Westling	832	10	Spectrum
Rhizoctonia solani Kühn	2355	3	Spectrum
Trichoderma viride	1700	Trace	Cut-plug
		0/14	Spectrum
Ustilago zeae (Beckm.) Ung.	2321	Trace	Cut-plug
		15	Spectrum
Verticillium albo-atrum Reinke et Berth.	1204	0/22	Spectrum
Fusarium oxysporum f. *lycopersici* +	1985 }		
Cladosporium herbarum	2175 {	20	Spray
Ophiostoma (*Ceratostomella*) *ulmi* +	2356 }		
Ustilago zeae	2321 {	40	Spray
Trichoderma viride +	1700 }		
Gibberella fujikuroi	2278 {	35	Spray

[a] Culture grown on M-1 agar (11). Results in most instances are representative of those obtained in several runs.
[b] Streptothricin resistant.
[c] Streptomycin resistant.
[d] Diagonal indicates partial inhibition with zone to the extent of the second number; complete inhibition occurred to extent of first number.

VATS.—Highest yields of the active factors, as measured by paper-disc assay with *T. viride*, *S. pastorianus*, and *B. subtilis*, were obtained in a medium composed of 3 per cent soybean meal, 2 per cent commercial glucose, and tap water in shaken-flasks. The medium was adjusted to pH 7.0 and sterilized for 30–40 minutes at 121.5° C. Fermentations were conducted for 96 hours at 28°–30° C on a Gump rotary shaker operating at 200 rpm. An extremely stable foam formed after about 72 hours of fermentation. The final pH range of shaken-flask fermentations was 7.0–8.0.

Fermentations conducted in 20-liter fermentors produced the same antibiotics as those obtained in shaken-flasks, but in different relative amounts. The most striking difference was a marked reduction in

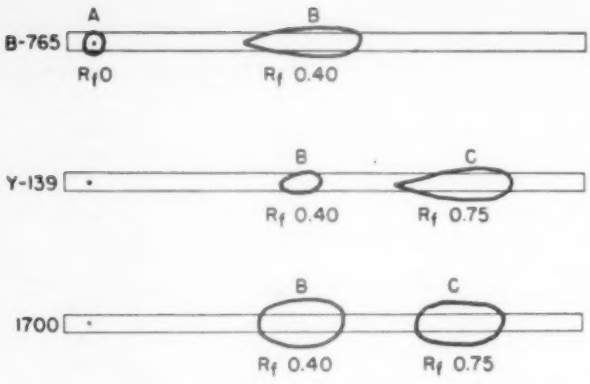


FIG. 2. Autobiographs of strain NRRL B-1699 culture filtrate in tests with *Bacillus subtilis* (NRRL B-765), *Saccharomyces pastorianus* (NRRL Y-139), and *Trichoderma viride* (NRRL 1700).

the antibacterial activity in the fermentors. The medium finally selected for use was composed of 2 per cent soybean meal, 0.5 per cent commercial glucose, and tap water. The pH was adjusted to 6.25 following sterilization. After the fermentation had progressed for 12 hours, the pH reached 6.9 and was held at pH 6.9–7.1 thereafter by an automatic pH control unit. Fermentations were carried out at 28° C for 12–20 hours with aeration at the rate of ½ v/v/minute and with agitation by a paddle-type stirrer operating at 300 rpm. A 25 per cent solution (v/v) of heptadecanol in absolute ethyl alcohol was added when required to control foaming.

Preliminary tests against active infections by the rust fungus in bean plants indicated that material obtained from vat fermentations had little antirust activity. Antirust activity was eventually obtained in the vat fermentors through use of the shaken-flask medium, elimination of the use of antifoam, agitation at 200 rpm, and aeration at the rate of ¼ v/v/minute through the top of the fermentors. With this procedure, up to 190 hours was required to complete a fermentation. Further vat fermentor studies are in progress.

PRELIMINARY CHEMICAL CHARACTERIZATION.—The active components in the culture filtrates of *S. cinnamomeus* f. *azacoluta* were studied by means of descending paper chromatography; the solvent system was a 6:4:3 mixture of 1-butanol, pyridine, and water. After development, the paper strips (Whatman No. 1) were air-dried and placed on agar plates seeded with either *T. viride*, *S. pastorianus*, or *B. subtilis*. Incubation at 30° C overnight (or longer if necessary) gave the autobiographs shown in Figure 2.

Three antibiotics were detected with the test organism as shown in Figure 2 and these have been designated as A, B, and C; some preliminary observations on these antibiotics are given below.

The stabilities of the antibiotics under various conditions were studied; both paper-disc assays and paper chromatography were used to determine the extent and nature of any inactivation. Antibiotics A and B were stable for 30 minutes at 90° C in the pH range 3–9, although there was considerable destruction of antibiotic C under these conditions. Culture liquors were lyophilized or spray-dried with no significant loss of antibiotics A, B, or C.

Passage of the culture liquor through a monobed resin column of Amberlite MB-3 did not remove antibiotics A or B, possibly an indication of their neutral character. The treatment, however, did result in some reduction in the amount of antibiotic C, owing either to instability of the antibiotic or to adsorption by the resin. The removal of only a portion of antibiotic C could be accounted for by its large molecular weight, as found in the dialysis experiments described below. Neither antibiotic A, B, nor C is extracted from culture filtrates by ether, chloroform, or ethyl acetate. Extraction with 1-butanol removed antibiotics B and C from the culture filtrate; however, concentration of the extract *in vacuo* gave a solid with almost no antibiotic C activity. The solid, which retained most of the original antibiotic B activity, was virtually inactive against bean rust in plants.

In dialysis experiments, the progress of which was followed by means of paper-disc assays and paper chromatography, it was found that antibiotics A and C did not dialyze[6], whereas antibiotic B was dialyzable. The antirust activity also appeared to dialyze, although only a part of this activity could be accounted for. On the basis of these preliminary experiments it appears that antibiotics A, B, or C singly are not responsible for the antirust activity. The development of a laboratory assay method for the antirust factor would expedite fractionation work and is in progress.

Although it has not yet been possible to establish the nature of the antirust activity, we have been successful in preparing from culture filtrates a spray-dried product that retains almost all of the antirust activity.

BIOLOGICAL CHARACTERISTICS.—Because of the promise shown by tests with culture filtrates, antibiotic spectrum studies with strain NRRL B-1699 were expanded. The results (Table 4) emphasize the wide range of activity manifested against both Gram-negative and Gram-positive bacteria Especially noteworthy is the antagonism displayed against the fungi.

FERMENTATION SECTION, NORTHERN UTILIZATION RESEARCH BRANCH
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
PEORIA, ILLINOIS

[6] Dialysis conducted with Nojax casing, The Visking Corporation, Chicago, Illinois.

LITERATURE CITED

1. ABBEY, A. 1952. Neomycin plate assay. Antibiot. and Chemother. **2**: 528-531.
2. ANDERSEN, A. A. 1951. A rapid plate method of counting spores of Clostridium botulinum. Jour. Bact. **62**: 425-432.

3. Benedict, R. G., W. Dvonch, O. L. Shotwell, T. G. Pridham, and L. A. Lindenfelser. 1952. Cinnamycin, an antibiotic from Streptomyces cinnamoneus nov. sp. Antibiot. and Chemother. **2**: 591-594.
4. Dubos, R. J., and G. Middlebrook. 1947. Media for tubercle bacilli. Amer. Rev. Tuberc. **56**: 334-345.
5. Duché, J. 1934. Les Actinomyces du groupe albus. Encyclopédie Mycologique. VI. Paul Lechevalier et Fils, Paris. 376 p.
6. Duggar, B. M., E. J. Backus, and T. H. Campbell. 1954. Types of variation in actinomycetes. Ann. N. Y. Acad. Sci. **60**: 71-85.
7. Dvonch, W., O. L. Shotwell, R. G. Benedict, T. G. Pridham, and L. A. Lindenfelser. 1954. Further studies on cinnamycin, a polypeptide antibiotic. Antibiot. and Chemother. **4**: 1135-1142.
8. Hesseltine, C. W., R. G. Benedict, and T. G. Pridham. 1954. Useful criteria for species differentiation in the genus Streptomyces. Ann. N. Y. Acad. Sci. **60**: 136-151.
9. Millard, W. A., and S. Burr. 1926. A study of twenty-four strains of Actinomyces and their relation to types of common scab of potato. Ann. Appl. Biol. **13**: 580-644.
10. Pridham, T. G., and D. Gottlieb. 1948. The utilization of carbon compounds by some actinomycetales as an aid for species determination. Jour. Bact. **56**: 107-114.
11. Pridham, T. G., L. A. Lindenfelser, O. L. Shotwell, F. H. Stodola, R. G. Benedict, C. Foley, R. W. Jackson, W. J. Zaumeyer, W. H. Preston, Jr., and J. W. Mitchell. 1956. Antibiotics against plant disease. I. Laboratory and greenhouse survey. Phytopathology **46**: 568-575.
12. Ridgway, R. 1912. Color standards and color nomenclature. Published privately by the author, Washington, D. C. 44 p., 53 plates with named color tabs.
13. Waksman, S. A. 1919. Cultural studies of species of Actinomyces. Soil Sci. **8**: 71-215.
14. Waksman, S. A., and H. A. Lechevalier. 1953. Guide to the classification and identification of the Actinomycetes and their antibiotics. Williams and Wilkins, Baltimore, Maryland. 246 p.
15. Whiffen, A. J. 1948. The production, assay, and antibiotic activity of actidione, an antibiotic from Streptomyces griseus. Jour. Bact. **56**: 283-291.

PHYTOPATHOLOGICAL NOTES

A Method for Observing Stomatal Penetration by Uredospore Germ Tubes of Puccinia graminis f. sp. tritici.[1] Lawrence J. Petersen.[2] Long and Clements[3] used collodion to study the number and distribution of stomata on plant surfaces. A unique application of this collodion-film impression technique, in which cellulose acetate was used as the impression medium, has been made recently.

Wheat leaves were inoculated with uredospores of *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. and the germ tubes then stained *in situ* by immersion of the leaves in 10 ml of lacto-phenol containing 0.5 per cent cotton blue (the staining process may be omitted if a phase contrast microscope is used). Excess moisture was allowed to evaporate from the leaf surface before the first coat of cellulose acetate was applied by means of a chromatography sprayer. After a 1-hour drying period, another heavier coat of the acetate was applied and allowed to dry for approximately 4 hours at 20°C. To facilitate removal of the dried acetate film, a portion of a cellophane tab was placed in juxtaposition at one end of the leaf and sprayed with cellulose acetate simultaneously with the leaf.

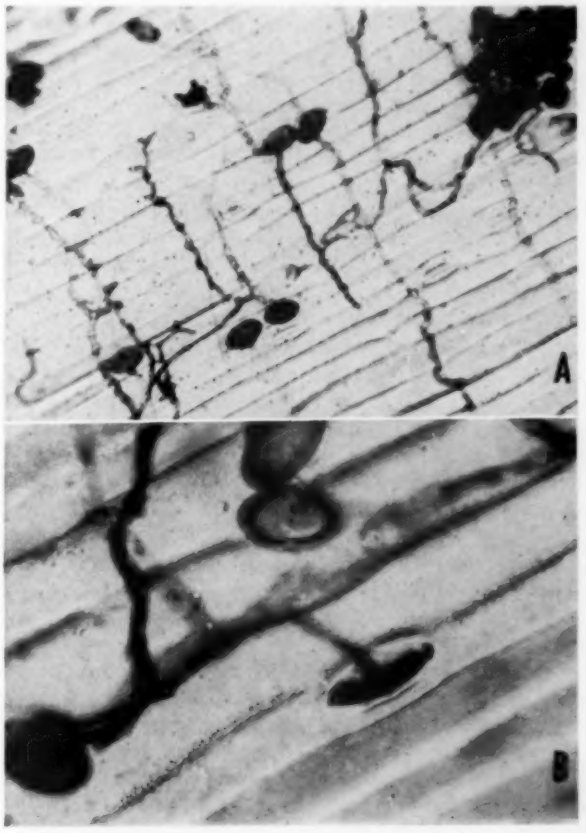


Fig. 1. Photomicrographs of stained cellulose acetate epidermal impressions. A) Germinated uredospores. (280×). B) Germinated uredospore of *P. graminis* f. sp. *tritici*, with germ tube that has penetrated a stomate of Marquis wheat. (840×).

[1] Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Series Paper No. 447. This work was supported in part by a contract (NR 131-216) between the Office of Naval Research and Colorado Agricultural and Mechanical College.

[2] Graduate Assistant in Research and Teaching, Colorado Agricultural and Mechanical College, Fort Collins, Colorado. Assistance from Dr. L. W. Durrell, Professor Emeritus, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, is gratefully acknowledged.

[3] Long, F. L., and F. E. Clements. 1934. The method of collodion films for stomata. Amer. Jour. Bot. 21: 7-17.

The cellulose acetate bearing the uredospores, germ tubes, and the impression of the epidermis and stomata was carefully stripped from the leaf surface, inverted, and placed on a slide. The strip of film could then be studied under the microscope, when convenient, and/or photographed.

By means of this method, it is possible to determine 1) the approximate number of germ tubes that actually penetrate the individual stomata, 2) the total number of spores per unit area of leaf surface, 3) germination percentage of spores on the leaf, 4) spore-stomate spatial relationships, and 5) other post-germination phenomena.—Colorado Agricultural Experiment Station, Fort Collins, Colorado.

Rediscovery of Sulfur as a Fungicide. S. E. A. McCallan. Following the use of sulfur as a fungicide by the ancients, the knowledge appears to have been lost until it was rediscovered in fairly recent times. Practically all texts credit the rediscovery of sulfur to John Robertson in 1824. More than a score of years ago a limited search revealed 2 references that pre-dated Robertson. A further search possibly would disclose earlier ones.

In 1802 there was published in London "A treatise on the culture and management of fruit trees . . . observations on the diseases, defects, and injuries . . . with an account of a particular method of cure. By William Forsyth, Gardener to His Majesty, &c. &c." On page 250 there is the following statement: "Whenever you apprehend danger, wash or sprinkle the trees well with urine and lime-water mixed; and when the young and tender shoots are much infected, it will be necessary to wash them well with a woollen cloth dipped in the following mixture, so as to clear them of all the glutinous matter, that their respiration and perspiration may not be obstructed.

"Take tobacco one pound, sulphur two pounds, unslaked line one peck, and about a pound of elder-buds; pour on the above ingredients ten gallons of boiling water; cover it close, and let it stand till cold; then add as much cold water as will fill a hogshead. It should stand two or three days to settle: then take off the scum, and it is fit for use."

In the Memoirs of the Caledonian Horticultural Society, Vol. 1, 1814, there is an article by David Weighton, "On destroying insects, and removing mildew, and canker in fruit trees." On page 135 he states, "The mildew may be kept under (I cannot say be cured), by washing trees affected by it, with a mixture of sulphur and lime-water. My mode of application is, by the garden engine, with a little soap, or any other thing that will separate it from the trees; this clears them at the time, but does not prevent the mildew from returning again.*

*"My friend Mr. John Hay of Edinburgh, having been very successful in preventing mildew, I shall here state his mode of management in his own words: 'Whenever mildew was observed to affect a leaf or the point of any shoot, the diseased leaf was picked off, the point of the shoot dipped in water in which black soap had been dissolved, and it was well drudged with a bellows-pluff, filled with sulphur, (sometimes mixed with Scots snuff.) This was generally done in the evening, and it was washed off with the engine or squirt, the following evening, if the state of the trees with respect to flowering or ripe fruit, did not forbid it. All the trees that were ever under my care, were thus prevented from being injured; but strict attention was always paid to the checking of the mildew at its first appearance'."

It is probable that many of the better informed gardeners were using sulfur at least by 1800, if not earlier; however, if an individual is to be named and until earlier reference can be found, the credit should go to William Forsyth for his report of 1802.—Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

Symptomatology of Bacterial Spot of Pepper and Tomato in Southern Florida.[1] R. S. Cox, Robert A. Conover, and Grover Sowell, Jr. Textbook symptomatology frequently is inadequate as a guide to the identification of plant diseases in southern Florida. For example, leafspot diseases usually characterized by the formation of discrete lesions many times appear in southern Florida fields as blights or scorches. This can be attributed to the fact that prolonged periods of humidity, moisture, and temperature optimum for disease development occur in this area. As these environmental factors become more favorable for disease development, the spot gradually enlarges or loses its identity entirely as more and more of the leaf blade is involved. Thus, it would appear that many "leafspot" diseases have been so named and described on the basis of arrested disease development in areas of less than optimum environment.

Bacterial spot (caused by *Xanthomonas vesicatoria* (Doidge) Dows.) serves as a typical illustration. Although leaf tip and marginal burn and leaf scorch of tomato have been described,[2,3] the occurrence and significance of these phases of the disease apparently have not been recognized in the United States. A description of such symptoms on pepper appears to be lacking. Since this unarrested development is a common phase of the disease in southern Florida and since its presence provides considerable confusion with respect to identity, a brief report on symptomatology is deemed desirable. The discussion will be restricted, in the main, to pepper, although similar symptoms are common to tomato.

[1] Contribution of the Everglades Experiment Station, the Sub-Tropical Experiment Station, and the Gulf Coast Experiment Station. Florida Agricultural Experiment Station Journal Series, No. 485.

[2] Doidge, Ethel M. 1921. A tomato canker. Ann. Appl. Biol. 7: 407-430.

[3] Sutton, W. S. 1948. Bacterial spot of tomatoes. Agr. Gaz. N. S. Wales 59: 595-597.

During periods of dry weather, either hot or cool, leaf symptoms are not unlike those generally associated with the disease (Fig. 1, A). During periods of continuous rainfall, heavy fog, or heavy dew, however,

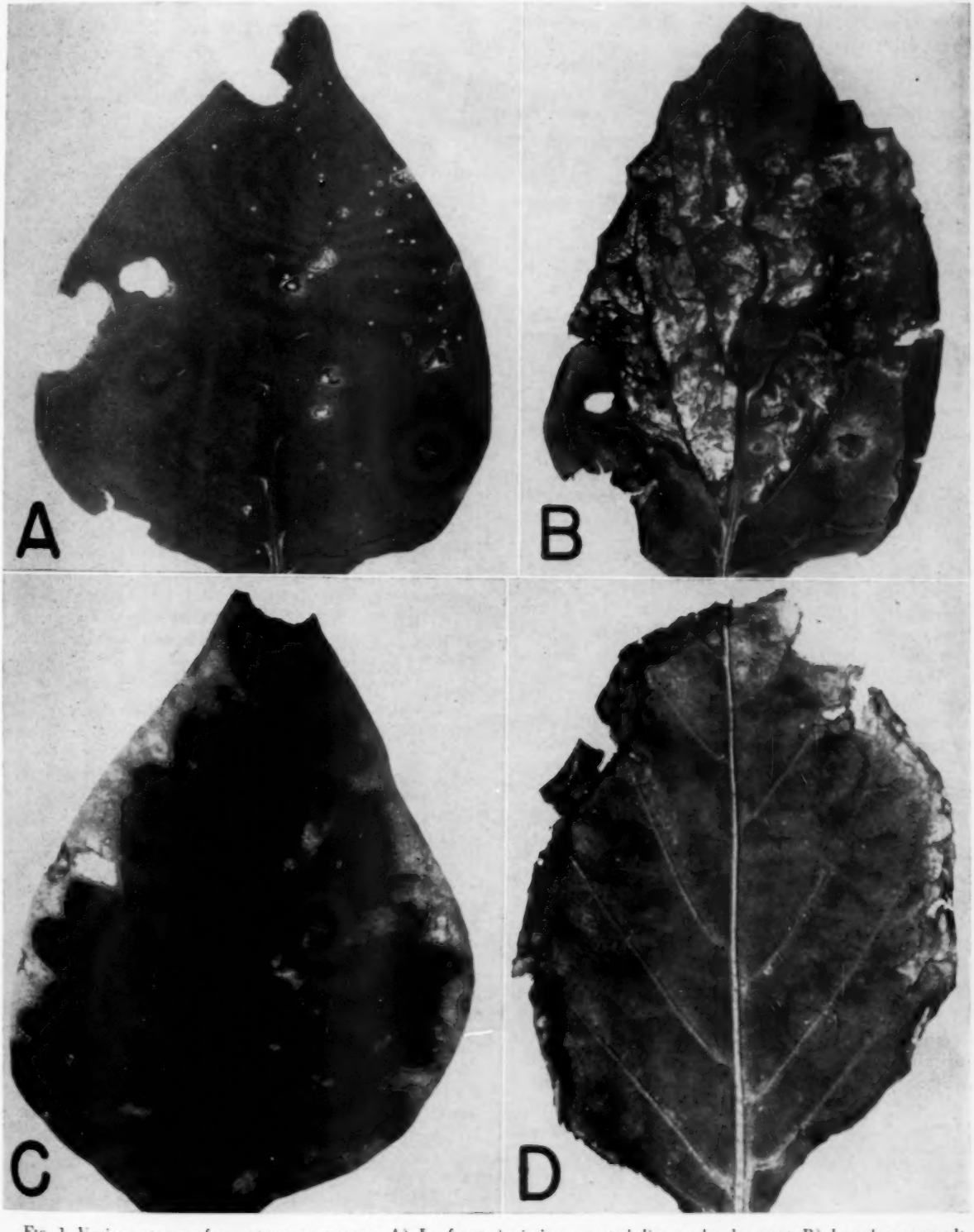


FIG. 1. Various stages of symptoms on pepper. A) Leafspot depicting arrested disease development. B) Leaf blight resulting from unarrested disease development. C) Chlorotic stage of marginal leaf infection. D) Necrotic stage of marginal leaf infection. Photographs by Henry M. Spelman III.

the leaves take on a blighted (Fig. 1, B) or scorched (Fig. 1, D) appearance rather than retaining the small well-defined spots generally associated with the disease. Observations indicate that water of guttation plays a role in the development of these symptoms. Thus, Higgins'[4] statement that large areas of the leaf are never killed, though undoubtedly correct for Georgia, is not only inaccurate for the disease as it occurs in southern Florida but is actually misleading.

Initial symptoms of these blighting phases of the disease appear as chlorotic areas, more often than not along the margin of the leaf, but occurring within the leaf blade as well (Fig. 1, C). The initial chlorotic stage rapidly progresses through a watersoaked, greasy appearance to one of extensive necrosis that is often mistaken for fertilizer burn (Fig. 1, D). Thus, the yellowed appearance of infected (lower) leaves that results from numerous infection centers (spots) and is characteristic of the the disease in more northerly latitudes is obscured by that of a "burned over" one due to severe leaf necrosis.

All stages of the symptoms described here have been artificially reproduced in the greenhouse where prolonged periods of air saturation and of free water on the leaves have been maintained. Everglades Experimental Station, Sub-Tropical Experiment Station, and Gulf Coast Experiment Station.

The Effects of Temperature on the Resistance to Oat Stem Rust Conditioned by the BC Genes.[1] B. J. Roberts and M. B. Moore.[2] In the breeding of oat varieties resistant to stem rust, the so-called "Canadian" or "Hajira" type of resistance recently has been used rather extensively. It is presumed to confer resistance at temperatures up to 82°–85°F to all known races of the stem rust fungus (*Puccinia graminis* f. sp. *avenae* Eriks. & E. Henn.) except race 7A, but at higher temperatures in the greenhouse, varieties that have only this type of resistance become completely susceptible to all races. Recently, Welsh[3] indicated that this type of resistance may be due to 2 linked genes, which he designated "BC." The specific objective of this investigation was to determine whether the resistance associated with the BC genes at 75°F and the susceptibility at 85°F are due to "local" or to "systemic" reaction in the plant.

For this work, seedling plants of oat (*Avena sativa* L.) varieties Rodney (C.I. 6661), Canuck (C.I. 2024), Victoria-Hajira-Banner (R.L. 1276), and (Victoria × Hajira-Banner) × Colo (C.I. 6537) were inoculated with race 7 of the oat stem rust fungus. The first 3 varieties have the BC genes, whereas (Victoria × Hajira-Banner) × Colo reacts to the races of this fungus as though it had the BC genes in combination with the White Russian gene for resistance. The White Russian gene conditions no resistance to race 7. Previous work indicates that where 2 or more genes controlling resistance to different races are present in a single variety, each exerts its influence independently; therefore the presence of the second gene should not affect the results of this study.

The culture of race 7 used in this study was obtained from the Cooperative Rust Laboratory at St. Paul and was considered to be typical for the race. A special temperature-control chamber was devised in which one part of a plant or even one part of a leaf blade could be kept at 85°F while the rest of the plant or the blade remained outside of the chamber at a greenhouse temperature of 75°F.

Seedlings of the 4 oat varieties were grown and inoculated at 75°F. They were then transferred to the temperature-control chamber, and some were placed so that the tip halves of some of the first leaf blades were at 75°F and the basal halves of the same blades were at 85°F. Conversely, other seedlings were placed so that the tip half of a first leaf blade was at 85°F and the basal half was at 75°F. Several plants of the 4 varieties were placed entirely outside the chamber at 75°F as check plants. The temperature of the air surrounding the plant part was recorded with mercury thermometers and with thermocouples. The high temperature varied from 80° to 90°F, with an average temperature of approximately 86°. The temperature of the greenhouse varied from approximately 70° to 80°F, with an average temperature of approximately 75°.

All 4 varieties were identical in their reaction to race 7. The rust reaction of a specific leaf segment was related directly to the air temperature to which it was exposed. All tips and basal portions kept at 75°F were resistant, whereas all tips and basal portions kept at 85°F were susceptible. There were no measurable transition zones of intermediate reactions between those portions of leaves subjected to different temperatures. Check plants kept at 75°F were resistant and check plants kept at 85°F were susceptible, indicating that the lower temperature was well within the range for the expression of resistance by genes BC and that the higher temperature was well within the range for susceptibility.

It was concluded from the results obtained in this study that the effects of different temperatures on the expression of resistance due to the BC genes in oats are localized in the specific parts of the plants that are subjected to each temperature. Since the effects are localized, it also was concluded that any substance or substances that may be responsible for resistance at 75°F or for susceptibility at 85°F either are not translocated or are translocated at a rate insufficient to affect rust development.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

[1] Paper No. 3461, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Agent, U. S. Dept. of Agriculture, Field Crops Research Branch, Agricultural Research Service, and Instructor, Department of Plant Pathology and Botany, University of Minnesota, respectively.

[3] Welsh, J. N., and T. Johnson. 1954. Inheritance of reaction to race 7A and other races of oat stem rust, Puccinia graminis avenae. Canad. Jour. Bot. 32: 347-357.